Gastrointestinal symptoms, nutritional status and small intestinal bacterial overgrowth in patients with cancer

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Gastrointestinal Symptoms, Nutritional Status and Small Intestinal Bacterial Overgrowth in Patients with Cancer

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Abstract

Oesophagogastric (OG) cancer patients are at risk of developing persistent gastrointestinal (GI) symptoms and/or malnutrition. It is possible that GI symptoms co-exist with malnutrition rather than simply occurring in isolation. Eighty patients with OG cancer were recruited to a prospective observational cohort study to explore this relationship at the point of diagnosis and at 3- and 12 months post-diagnosis (Chapter 3). At 12 months, GI symptoms and malnutrition persisted or developed in 71.9% and 59.6% respectively. High symptom burden tended to be associated with poorer nutritional status and low symptom burden tended to be associated with better nutritional status at each time point.

An effective nutritional screening tool is essential for detecting malnutrition in the OG oncology setting. A validation study of the Malnutrition Universal Screening Tool against an accepted standard (Patient Generated Subjective Global Assessment) was undertaken (Chapter 4). The screening tool had a sensitivity of 61% and a specificity of 74% and thus, is not suitable for use in this setting.

Theoretically, as a consequence of the treatments received, patients with cancer are at high-risk for the development of small intestinal bacterial overgrowth (SIBO), a condition that implies abnormal bacterial colonisation of the proximal small bowel. The incidence of SIBO after diagnosis was measured in a sub-group of the OG cancer cohort (n= 17) and was found to be 82.4% (Chapter 3).

There is no gold-standard test for SIBO and a new, accurate diagnostic tool would represent a major development. A cohort of 200 patients previously treated for cancer and undergoing testing for suspected SIBO were recruited (Chapter 5). The metabolic profile of their urine samples was assessed (using metabolomics technology) to establish whether any metabolite(s) could separate individuals with and without SIBO. N-acethylglutamine, a modified amino acid, showed some ability to separate the two groups.

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Glossary of Abbreviations

ANOVA	Analysis of Variance
ASPEN	American Society for Parenteral and Enteral Nutrition
AUC	Area Under Curve
BAPEN	British Association of Parenteral and Enteral Nutrition
BDA	British Dietetic Association
BMI	Body Mass Index
CCK	Cholecystokinin
CFU/ml	Colony-Forming Units Per Millilitre
CH_4	Methane
CI	Confidence Interval
DAUGS20	Dysfunction After Upper Gastrointestinal Surgery Tool
EAR	Estimated Average Requirement
ECF	Epirubicin, Cisplatin and Fluorouracil
ECOG	Eastern Cooperative Oncology Group
ECX	Epirubicin, Cisplatin and Capecitabine
EN	Enteral Nutrition
EORTC	European Organisation for the Research and Treatment of Cancer
EPIC	European Prospective Investigation into Cancer (in Norfolk)
EPR	Electronic Patient Record
ESPEN	European Society for Clinical Nutrition and Metabolism
FACT-G	Functional Assessment of Cancer Therapy-General
FETA	FFQ EPIC Nutrition Tool for Analysis
FFQ	Food Frequency Questionnaire
GHMBT	Glucose Hydrogen Methane Breath Test
GI	Gastrointestinal
GOJ	Gastro-oesophageal Junction
GSRS	Gastrointestinal Symptom Rating Scale
^{1}H NMR	Hydrogen Nuclear Magnetic Resonance (Spectroscopy)

IBS	Irritable Bowel Syndrome
IQR	Interquartile Range
KCL	King's College London
LCA	London Cancer Alliance
MAG	Malnutrition Advisory Group
MDT	Multidisciplinary Team
MNA	Mini Nutritional Assessment
MST	Malnutrition Screening Tool
MSTC	Malnutrition Screening Tool for Cancer
MUST	Malnutrition Universal Screening Tool
NMR	Nuclear Magnetic Resonance (Spectroscopy)
NOESY	Nuclear Overhauser Effect Spectroscopy
NRI	Nutrition Risk Index
NRS	Nutrition Risk Screening (2002)
OG	Oesophagogastric
OGD	Oesophago-gastroduodenoscopy
ONS	Oral Nutritional Supplement
OR	Odds Ratio
PC	Principle Component
PCA	Principle Component Analysis
PG-SGA	Patient Generated Subjective Global Assessment
PLSDA	Partial Least Squares Discriminatory Analysis
Ppm	Parts Per Million
QLQ-C30	Quality of Life Questionnaire-Core 30
QLQ-OES18	Quality of Life Questionnaire: Oesophageal-Specific Module
QLG-OG25	Quality of Life Questionnaire: Oesophagogastric-Specific Module
QLQ-STO22	Quality of Life Questionnaire: Gastric-Specific Module
QoL	Quality of Life
RM	The Royal Marsden

RNI	Reference Nutrient Intake
ROC	Receiver Operating Characteristic (Curve)
SD	Standard Deviation
SGA	Subjective Global Assessment
SIBO	Small Intestinal Bacterial Overgrowth
Sp.	Species
SPSS	Statistical Package for the Social Sciences
TNM	Tumour Node Metastasis
TOCSY	Total Correlation Spectroscopy
UK	United Kingdom
US	United States
Vs	Versus

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Chapter 1

Introduction

1.1 Gastrointestinal Symptoms, Nutritional Status and Food Intake in Patients with Oesophagogastric Cancer

1.1.1 Classification, Incidence, Management of Oesophagogastric Cancer

Oesophageal cancer, cancer of the gastro-oesophageal junction (GOJ) and gastric cancer are collectively referred to as oesophagogastric (OG) cancer. Patients with OG cancer are often classified into five groups according to the site and histology of their tumour (The Royal College of Surgeons of England 2013) corresponding to:

- Squamous cell carcinomas of the oesophagus
- Adenocarcinomas of the upper and middle oesophagus
- Adenocarcinomas of the lower third of the oesophagus and Siewert type I tumours
- Siewert type II and type III tumours
- Tumours of the stomach

The three-category Siewert classification system is commonly used to describe the tumours of the GOJ, as follows (Siewert & Stein 1998):

- Type I: Adenocarcinoma of the lower third of the oesophagus which usually arises from an area with specialised intestinal metaplasia of the oesophagus (i.e. Barrett's oesophagus) and which may infiltrate the GOJ from above
- Type II: True carcinoma of the cardia arising from the cardiac epithelium or short segments with intestinal metaplasia at the GOJ
- Type III: Subcardial gastric carcinoma that infiltrates the GOJ and lower third of the oesophagus from below

For OG tumour staging, the Tumour Node Metastasis (TNM) Classification is an anatomically based system that records the primary and regional nodal extent of the tumour and the absence or presence of metastases. Each individual aspect of TNM is termed a *'T'* category (that describes the primary tumour site), a *'N'* category (that describes the regional lymph node involvement) and a *'M'* category (that describes the presence or otherwise of distant metastatic

spread). In 2009 the Union for International Cancer Control in collaboration with the American Joint Committee on Cancer published TNM 7th edition, which has become the standard for describing and categorising OG cancer stages and progression in the United Kingdom (UK) (Sobin et al. 2009; Allum et al. 2011). Using this, assuming an assessment of the cancer can be undertaken, there are four sub-groups in the T category (T1, T2, T3, T4), three in the N category (N1, N2, N3) and one in the M category (M1). Further detailed information on the classification of oesophageal, GOJ and gastric cancer is shown in Appendix 8.1. Of note, tumours including the oesophagus and within 5 cm of the GOJ are classified as oesophageal cancers and all others are classified as gastric cancer. As such, the majority of cancers in the GOJ region are considered gastric in nature and therefore incidence statistics often report GOJ and gastric cancers together.

The precursor of squamous cell carcinoma is epithelial dysplasia, which progresses to carcinoma and then to invasive carcinoma. Causal factors for squamous cell carcinoma of the oesophagus include alcohol, tobacco, caustic injury, previous radiotherapy treatment for head and neck or breast cancer, low intake of fruits and vegetables and high intake of processed meat/N-nitroso compounds (Navarro Silvera et al. 2011; Napier et al. 2014; Enzinger & Mayer 2003). Adenocarcinoma occurs when the squamous mucosa undergoes metaplasia into specialised columnar epithelium and then becomes dysplastic. Causal factors for adenocarcinoma of the GOJ include gastro-oesophageal reflux disease, obesity, smoking, low intake of fruits and vegetables and high intake of processed meat/N-nitroso compounds, while for adenocarcinoma of the stomach, causal factors include *H. Pylori* infection, atrophic gastritis, smoking, low intake of fruits and vegetables and high intake of processed meat/N-nitroso compounds (Sehdev & Catenacci 2013; Steevens et al. 2010; Navarro Silvera et al. 2011; DeVita et al. 2012; Trivers et al. 2008; Shimazu et al. 2014).

The past decade has seen changes in the epidemiology of OG cancer with an increase in the incidence of GOJ adenocarcinomas. In fact, in England, adenocarcinomas are now the most common histological type of OG cancer (Cancer Research UK 2014). This probably reflects the

effect of chronic gastro-oesophageal reflux disease and the increasing incidence of obesity (Wu et al. 2003; Lindblad et al. 2005). In the UK, this increase in incidence is more rapid than that reported by any other country in the world (Bollschweiler et al. 2001).

Worldwide incidence statistics for 2012 estimate that oesophageal and GOJ/gastric cancers were the 8th and 5th most common cancers, making up 3% and 7% of all cancers respectively. Similarly, oesophageal and GOJ/gastric cancers were the 8th and 5th most common cause of mortality in this year (International Agency for Research in Cancer 2014). In the UK in 2011, there were 8,332 new cases of oesophageal cancer and 7,089 new cases of GOJ/gastric cancer (Cancer Research UK 2014). In addition, oesophageal and GOJ/gastric cancers were the 8th and 11th most common types respectively for males and the 13th and 14th most common types for females.

Overall OG cancer is twice as common in males as in females but this male predominance is particularly strong for GOJ/gastric cancer at 7:1 (Devesa et al. 1998; Parkin 2001; The Royal College of Surgeons of England 2013). The median age at time of diagnosis of OG cancer is 74 years for all types combined, with 45% of cases being detected in those aged 55-74 years (Coupland, Allum, et al. 2012a).

The incidence rates of these cancers in broad ethnic groups have been studied. White males and Bangladeshi females have the highest incidence of oesophageal cancer, while Pakistani males and females have the lowest incidence. For GOJ/gastric cancer, Black Caribbean males and females have the highest incidence and Indian males and females have the lowest incidence compared with their White counterparts (Coupland, Lagergren, et al. 2012b).

There are guidelines for the referral of patients with suspected upper-gastrointestinal (GI) cancer (National Institute for Health and Clinical Excellence, Great Britain 2005). These guidelines recommend that an urgent referral for endoscopy assessment or to a specialist with expertise in upper-GI cancer should be made for patients aged 55 years and older with

unexplained and persistent recent-onset dyspepsia. An urgent referral should also be made for patients of any age with dyspepsia who present with any of the following *'alarm features'*: chronic GI bleeding, dysphagia, progressive unintentional weight loss, persistent vomiting, iron deficiency anaemia, epigastric mass or suspicious barium meal result.

However, it has been demonstrated that use of *'alarm features'* alone to prompt the referral of patients under 55 years causes patients with early disease to be overlooked (Bowrey et al. 2006). For this reason, the diagnosis of upper-GI cancer as a result of an emergency admission still accounts for 15% of cases. The proportion diagnosed during emergency admission is higher for GOJ/gastric cancer than oesophageal cancer and the difference may be due to the fact that early symptoms of oesophageal cancer are easier to recognise (e.g. dysphagia and odynophagia), while GOJ/gastric cancer tends to present later with less specific signs and symptoms (e.g. early satiety, anaemia and weight loss), many of which are present in large numbers of individuals without cancer (The Royal College of Surgeons of England 2013).

Staging investigations for OG cancer should be coordinated within an agreed pathway led by an OG specialist multidisciplinary team (MDT) at a specialist centre. The core team specific to this MDT should include: two or more surgeons, a physician gastroenterologist, a clinical oncologist, a medical oncologist, a histopathologist, an imaging specialist, an OG nurse specialist, a core member of the specialist palliative care team, a dietitian specialising in OG cancer, an oncology specialist physiotherapist and a MDT coordinator/secretary (London Cancer Alliance 2014). Treatment recommendations should be undertaken in the context of this OG specialist MDT, taking into account patient co-morbidities, nutritional status, performance status, patient preferences and TNM staging information as per grade C evidence (Allum et al. 2011). Recommendations for the radical treatment (treatment with curative intent) of OG cancer are shown in Table 1-1. Patients considered for radical therapy are generally younger at diagnosis, have a better performance status and have fewer than two comorbidities compared with those treated palliatively with T4 disease (The Royal College of Surgeons of England 2013).

The surgical resection of the tumour remains the cornerstone of radical treatment for most OG cancers, consisting of oeophagectomy, oesophagogastrectomy or gastrectomy, as appropriate. For tumours of the oesophagus, an oesophagectomy is performed. In the UK, 97% of oesophagectomies are performed via the transthoracic approach and the remaining 3% are done via the transhiatal approach. The three transthoracic approaches are the two-phase Ivor Lewis, the three-phase McKeown and the left thoraco-abdominal approach. Gastrointestinal continuity is usually achieved by using the stomach remnant as a conduit with an end-to-side anastomosis. For tumours of the GOJ, a transhiatal extended total gastrectomy (oesophagogastrectomy) is the procedure used, often with the two-phase Ivor Lewis method and with GI continuity achieved as for oesophagectomy. For gastric resections, 87% of procedures are total or distal subtotal gastrectomies (The Royal College of Surgeons of England 2013).

Table 1-1 Expert recommendations for the radical treatment of oesophagogastric cancers in the United Kingdom

Cancer site	Histology	Conditions	Definitive treatment modalities
Upper oesophagus	SCC	T1, T2 or T3 disease	Chemoradiation
Middle and lower oesophagus	SCC	T1, T2 or T3 disease, good performance status and good exercise tolerance (if for surgery)	Chemoradiation alone or with surgery
Oesophagus and Siewert type I, II or III	AC	T1, T2 or T3 disease, good performance status and good exercise tolerance	Combined perioperative chemotherapy (pre- and postoperative) and surgery or Preoperative chemotherapy/ chemoradiation and surgery
Stomach	AC	T1, T2 or T3 disease, good performance status and good exercise tolerance Did not receive preoperative chemotherapy and at high risk of recurrence	Combined perioperative chemotherapy (pre- and postoperative) and surgery Surgery and postoperative chemoradiation
Table adapted from *'Guidelines for the management of oesophageal and gastric cancer'* (Allum et al. 2011). Abbreviations: SCC, squamous cell carcinoma; AC, adenocarcinoma.			

Partial gastrectomy is considered adequate when 5 cm clearance of the tumour is possible and this is usually the case for cancers of the antrum, but a total gastrectomy is usually necessary for cancers of the body and cardia. After a distal subtotal gastrectomy, reconstruction involves a Polya or Bilroth I procedure. Reconstruction after a total gastrectomy is generally performed using a Roux-en-Y loop with a retro-colic jejunal roux loop.

Resection may be combined with perioperative chemotherapy and/or radiotherapy. Definitive treatment for squamous cell carcinoma of the oesophagus is chemoradiation, usually with a radiation dose of 50.4 Gray in 28 fractions (London Cancer Alliance 2014). At present, the recommended chemotherapy regimens for oesophageal and GOJ tumours are: ECF (epirubicin, cisplatin and fluorouracil); or ECX (epirubicin, cisplatin and capecitabine). Tumours of the stomach are treated with cisplatin and fluorouracil or capecitabine (London Cancer Alliance 2014). By adding radiotherapy to chemotherapy there is a beneficial synergistic effect.

1.1.2 Survival and Quality of Life in Patients with Oesophagogastric Cancer

Following an oesophagectomy or gastrectomy, the median length of stay in hospital is 13 and 11 days respectively (The Royal College of Surgeons of England 2013). In the UK, the 90-day mortality rates for oesophagectomy and gastrectomy are 1.4% and 3.1% respectively and the expected recovery period is at least one year (Olsson et al. 2007; The Royal College of Surgeons of England 2013). The most recent Cancer Research UK statistics indicate that the one-year survival for oesophageal and GOJ/gastric cancers is 40% and 42% respectively. Long-term survival is mostly stage dependent (King et al. 1987; Vigneswaran et al. 1993). Overall five-year survival has gradually improved, although it remains low at 13% and 18% for oesophageal and GOJ/gastric cancers respectively, when all treatment modalities are combined (Cancer Research UK 2014). Specifically, for surgical patients, the five-year survival rates are higher at 27% and 28% respectively (Anderson et al. 2011).

Surgery, chemotherapy and radiotherapy are used to cure, prolong life and relieve symptoms in patients with OG cancer. However, these treatments often have both short- and long-term adverse effects that result in a deterioration in quality of life (QoL). To be able to distinguish between QoL in the broader sense and QoL connected with a patient's health, the concept of health-related QoL has been developed. This refers to the '*subjective evaluation of one's ability to perform usual tasks and their impact on one's everyday physical, emotional and social well-being*'. In this thesis, the term QoL is used when referring to health-related QoL (Fayers & Machin 2007).

Up until the late 1990's, morbidity and mortality were the main (and often the only) outcome measures used to evaluate the success of treatment for OG cancer. High morbidity and low survival rates associated with these cancers, especially oesophageal cancer, resulted in little interest in the measurement of QoL in research. However, in the past 15 years, there has been an improvement in treatment options and increasing numbers of long-term survivors. Also, QoL has been shown to be an independent predictor of survival after treatment for upper-GI cancer (Blazeby et al. 2001; Fang et al. 2004). Therefore, QoL has become increasingly accepted as an important outcome measure in trials. As such, a number of tools have been developed to measure QoL and can be classified into three main categories: generic tools, symptom-focused questionnaires and cancer-specific tools (Conroy et al. 2006).

The use of cancer-specific tools has become increasingly popular e.g. the Functional Assessment of Cancer Therapy-General (FACT-G) tool and the European Organisation for the Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire-Core 30 (QLQ-C30). The EORTC QLQ-C30 is a valid and reliable tool that has 30 items with five functional scales, three symptom scales and one global scale (Aaronson et al. 1993). It is applicable to all patients with cancer. In addition, site-specific modules have been developed: they include specific symptoms relevant to a cancer site and this increases the tool's sensitivity to detect small, but clinically important differences in QoL. It also allows the contribution of symptoms to overall QoL to be determined. For EORTC QLQ-C30, the oesophageal-specific module is QLQ-OES18 (18

additional items), the gastric-specific module is QLQ-STO22 (22 additional items) and the module for the oesophagus, GOJ and stomach combined is QLQ-OG25 (25 additional items) (Blazeby et al. 2003; Blazeby et al. 2004; Lagergren, Fayers, et al. 2007b).

It has been demonstrated that patients with newly diagnosed oesophageal cancer waiting for surgery have compromised QoL (Visser et al. 2006). This impairment in QoL continues, as patients who have undergone chemoradiation or surgery for OG cancer have reduced QoL during and after treatment (Blazeby et al. 2000; Carey et al. 2011; McLarty et al. 1997; Gillham et al. 2008). A prospective study of 56 patients undergoing definitive chemoradiation to the oesophagus found that QoL scores (using the EORTC tools) did not improve significantly one year after the end of treatment, though there was a slow improvement in emotional, cognitive and social scales over time (Gillham et al. 2008). Similarly, longitudinal and population-based studies have shown that oesophagectomy has an immediate negative impact on all aspects of QoL and there is a limited and slow recovery (Blazeby et al. 2000; Viklund et al. 2006).

Indeed, following oesophagectomy, those patients still alive at three years reported persistent impairment in QoL and those not living beyond 12 months did not regain preoperative QoL levels before their death, as measured by EORTC QLQ-C30 and QLQ-OES18 (Djärv et al. 2008; Lagergren, Avery, et al. 2007a). Interestingly, in their non-randomised study, using the same QoL tools, Ariga et al. directly compared the outcomes after treatment in patients with oesophageal cancer who had chosen to receive definitive chemoradiation (with salvage surgery if disease progressed) and those who had chosen to undergone surgery alone (Ariga et al. 2009). Quality of life was assessed by a cross-sectional survey in those patients who had survived at least two years. Results indicated that QoL was similarly compromised in both groups of patients except for symptoms such as diarrhoea, appetite loss, and eating problems, which were not as bothersome in the chemoradiation group compared with the surgical group (Ariga et al. 2009).

There are very few studies that have prospectively and critically assessed the QoL of gastric cancer patients, with the monitoring of change over time. However, the available literature suggests that for patients undergoing gastrectomy, QoL is impaired immediately postoperatively but appears to improve over time (Avery et al. 2010; Karanicolas et al. 2013; Conroy et al. 2006). In the study undertaken by Karanicolas et al., most patients returned to baseline QoL by approximately six months post-gastrectomy, although about one third continued to have important QoL impairment (Karanicolas et al. 2013). A limitation of this study is the large amount of missing data: 14% of patients were excluded because of this issue. In Avery's cohort, patients surviving two years generally recovered most aspects of QoL within six months of surgery and thereafter only reported problems with a few specific symptoms (Avery et al. 2010). However, 20% of patients died within six months of surgery during which QoL recovery was not achieved. Although this was a prospective and longitudinal study, the sample size was small (n= 58) which limits the interpretation of the data.

A prospective longitudinal Korean study that consecutively followed 465 newly diagnosed gastric cancer patients planned for radical resection is one of just a few large-scale studies designed to measure differences in QoL scores between surgical techniques (Kim A. R et al. 2012a). Quality of life was measured using EORTC QLQ-C30 and QLQ-STO22. The number of patients undergoing subtotal gastrectomy and total gastrectomy were 377 and 88, respectively. There was 119 also receiving chemotherapy and 39 receiving radiotherapy. Global QoL and emotional functioning was found to improve over time for the 12-month study period regardless of surgical technique. Patients in the subtotal gastrectomy group were more likely to report better QoL than those in the total gastrectomy group. Of note, fatigue, body image disturbance, impaired cognitive function and GI symptoms (such as diarrhoea, dysphagia and eating restrictions) were the unrecovered problems in both surgical groups. Patients in the total gastrectomy group were more likely to be diagnosed with T2 and T3 cancer and to receive chemotherapy and radiotherapy than those in the subtotal gastrectomy group. Even after adjusting for this, the QoL of the former was still worse than the latter (Kim A. R et al. 2012a).

The study has strengths with regard to the high numbers of patients followed up (85% at 3 months and 88% as 12 months) and the use of the standardised and valid EORTC tools.

An earlier study undertaken by a Swedish group supports the findings of this study. Svedlund and colleagues used the Gastrointestinal Symptom Rating Scale (GSRS) to assess GI symptoms in patients following total gastrectomy and subtotal gastrectomy for up to five years after surgery (n= 64) (Svedlund et al. 1999). They reported that patients who underwent total gastrectomy continued to suffer from a persistent decrease in QoL due to the presence of GI symptoms, especially indigestion and diarrhoea, whereas those who underwent subtotal gastrectomy experienced improvement in the same symptoms.

It is apparent, therefore, that regardless of tumour type or the treatment modalities received, OG cancer patients suffer impairment in their QoL, which does not always improve even months or years after the completion of treatment.

1.1.3 Gastrointestinal Function in Patients with Oesophagogastric Cancer

Gastrointestinal function in patients with OG cancer can be acutely and chronically affected by both the cancer itself and the oncological treatments received (Andreyev et al. 2011). With regard to the former, the tumour, especially when bulky, may cause a physical obstruction in the lumen of the GI tract, leading to symptoms directly. A tumour in the oesophagus can prevent the normal passage of food and fluid into the stomach causing dysphagia and/or odynophagia; a tumour at the cardiac sphincter muscle can stop the sphincter from working and lead to acid reflux/indigestion; a tumour in the pyloric region of the stomach can cause gastric outflow obstruction and result in nausea, vomiting, abdominal bloating/discomfort and constipation. In advanced disease, peritoneal spread may cause ascites or intestinal dysmotility, which can lead to small bowel obstruction and subsequent symptoms (e.g. abdominal bloating/discomfort and constipation).

Symptoms may also arise because of changes in GI physiological processes following oncological treatment. Cytotoxic cancer chemotherapy affects cellular cycles and proves particularly detrimental to rapidly proliferating cells such as enterocytes leading to inflammation, oedema, ulceration and atrophy (Beck et al. 2006). The GI mucosa is highly vulnerable to radiation therapy as it induces death in cells undergoing mitosis. As such, for those patients who undergo radiotherapy to OG tumours, there is a risk of complications to normal tissues around the tumour. In addition, the development of functional disorders after oesophagectomy or gastrectomy is related to the new anatomic and functional configuration of the upper-GI tract following surgery.

When radiotherapy is concomitant with chemotherapy, it is likely that the GI toxicity intensifies. However, randomised controlled trials in OG cancer comparing chemoradiation with chemotherapy alone focus on overall survival and disease-free survival, not on the prevalence and severity of GI symptoms, and so firm conclusions on this cannot be drawn (Stahl et al. 2009; Burmeister et al. 2011). There is an array of GI symptoms that can result from the anatomical changes and/or enterocyte damage caused by OG cancer treatment.

1.1.3.1 Gastrointestinal Symptoms in Patients with Oesophagogastric Cancer

The acute and chronic GI side effects of chemotherapy and radiotherapy in OG cancer have not been studied systematically or prospectively. There have been few studies assessing GI function following chemoradiation in patients with oesophageal cancer. In the few studies that have, most measured just a few GI symptoms or just those related to swallowing function.

For example, a study was undertaken in the United States (US) in the 1980's and early 1990's to determined the impact of chemoradiation on swallowing function in patients with oesophageal cancer (Coia et al. 1993). Pre-treatment and on-treatment swallowing was measured retrospectively using clinical notes, where the O'Rourke scale was used to determine the prevalence and severity of dysphagia (O'Rourke et al. 1988). The pre-treatment swallowing function of 120 patients was reported and 93% of them reported dysphagia: eating solids with

some dysphagia in 48 (40%); eating soft or pureed food in 50 (42%); drinking liquids only in 8 (7%); and no swallowing at all in 5 (4%). Sufficient information was present in the charts to determine the initial improvement in dysphagia in 102 patients and 90 (88%) of these experienced some degree of improvement in their dysphagia during treatment. After two weeks of treatment, 41 (45%) experienced improved swallowing, and at treatment completion (six weeks), 75 (83%) had improved. For those who had improvement, the median time to initial improvement was two weeks, and by four weeks, improvement was noted in 86% of patients. The range of time for patient improvement was 1-21 weeks. Although dysphagia was the only symptom measured in this study, it does provide interesting data for an oesophageal cancer cohort: a high prevalence of dysphagia at baseline and a rapid and considerable initial improvement in nearly 90% of those treated with chemoradiation.

Other trials recorded some GI symptoms in chemotherapy/chemoradiation treated patients using a QoL tool. For instance, the study undertaken by Blazeby et al., which examined QoL during neoadjuvant chemotherapy/chemoradiation for oesophageal carcinoma (Blazeby, Sanford, et al. 2005b). Quality of life was measured by EORTC QLQ-C30 and QLQ-OES18 and these tools provided information on GI symptoms experienced pre-treatment, seven weeks after the start of chemotherapy and five weeks later for patients undergoing chemoradiation. Thirty-four patients underwent chemoradiation and 48 received chemotherapy alone. With reference to specific GI symptoms, both the chemoradiation group and the chemotherapy group reported worsening of the following symptoms at seven weeks compared with baseline: nausea and vomiting ($p< 0.01$, $p= 0.05$), anorexia ($p< 0.01$, $p= 0.01$), diarrhoea ($p< 0.01$, $p= 0.01$) and taste changes ($p< 0.01$, $p< 0.01$). For chemoradiation patients, the symptoms of dysphagia, eating problems, and reflux that had relieved or been unchanged at week seven, had deteriorated by week 12 ($p= 0.03$, $p= 0.03$ and $p< 0.01$) compared with baseline. Also at 12 weeks, anorexia ($p= 0.03$) and diarrhoea ($p= 0.02$) continued to be significantly worse than baseline levels.

A similar study showed that the presence of GI symptoms resulted in a reduction in QoL following chemoradiation for oesophageal cancer (Avery et al. 2007). The aim of this 132-

patient study was to evaluate QoL during potentially curative chemoradiation (n= 51) and to compare this with QoL during combination treatment including surgery (n= 81). The same EORTC QoL measures were used as in Blazeby's study, with the following time points: pre-treatment, after induction chemotherapy (six weeks), at 12 weeks, and at six and nine months, when recovery was anticipated. Patients who had chemoradiation experienced a moderate reduction in QoL during treatment, with worsening of anorexia, taste problems, dry mouth, diarrhoea, nausea and vomiting (p-values not reported), although dysphagia significantly improved (p= 0.003). By nine months, their QoL levels had recovered and GI symptom severity had returned to pre-treatment levels, or had improved.

Although research into GI symptoms before and during chemotherapy or chemoradiation is limited, those available data indicate that symptoms may be common, certainty in the acute setting. There has been a much greater interest in measuring GI symptoms related to surgery in OG cancer. A seminal paper by Visick in 1948 outlined symptoms among 500 patients who had undergone gastrectomy for peptic ulcer disease (Visick 1948). Reported symptoms six months after surgery included pain, fullness, vomiting and diarrhoea. Although, the patients did not have cancer, this study was one of the first to highlight the burden of symptoms after radical upper-GI surgery.

In a cross-sectional study of 30 patients (at least six months) following major upper-GI surgery, Carey et al. used a stepwise backward linear regression model to assess which features (including nutritional status, dietary intake and GI symptoms) might explain QoL scores (Carey et al. 2011). All participants with the exception of one had surgery for a malignant tumour. Gastrointestinal symptoms were assessed using the GSRS tool. Quality of life was measured using the EORTC QLQ-C30. Total GSRS scores indicated that patients experienced mild symptoms, although the prevalence of individual symptoms was not reported. In the final model, only GSRS scores and nutritional status category were significant in explaining 50.3% of variance in QoL, ($F= 13.646$; $p< 0.001$). The GSRS measure recorded a high beta value of -0.441 ($p= 0.004$). This study is one of few to measure the association between GI symptoms

and QoL (Carey et al. 2011). Although, the sample size was small, these data highlight the detrimental effect that ongoing GI symptoms can have on QoL in this group. Further aspects of this study will be discussed in due course.

Many of the large prospective studies in OG cancer patients do not measure GI symptoms in isolation but rather use a QoL tool with one or multiple symptom scales within it, which can capture the influence of symptoms on overall QoL. For example, a Swedish research group carried out a prospective nationwide population-based study with the aim of assessing QoL six months after radical surgery for oesophageal cancer (Viklund et al. 2006). Two hundred and eighty-two postoperative patients and 3,069 Swedish natives completed the EORTC QLQ-C30 (includes five GI symptom measures) and QLQ-OES18 (includes 18 oesophageal-specific symptoms, 14 are GI in nature). With particular reference to the GI symptom results from this study, nausea and vomiting were significantly more common in people following oesophagectomy than in the general reference population ($p < 0.001$). Among the general symptom items, appetite loss and diarrhoea were dominating problems that were considerably more prevalent in OG cancer patients, particularly appetite loss ($p < 0.001$). For the oesophageal-specific symptoms, eating problems was the single worst symptom. Oesophageal pain, dysphagia and taste issues were also reported very frequently by the OG cancer patients (Viklund et al. 2006). With regard to the methodological aspects of this study, the population-based design reduced the risk of selection bias, while the large sample size diminished the risk of chance errors. Moreover, the use of QoL questionnaires with documented good reliability and validity was another one of its strengths.

A prospective study evaluated GI symptoms at two weeks, six months and 12 months post-oesophagectomy in 48 patients (Ludwig et al. 2001). A sub-group of these patients ($n = 10$) had also received chemoradiation. Periodic dysphagia was present in 52% of patients in the early postoperative period and at 12 months this symptom was reported by 38%. At the latter follow-up, 19% of patients still reported periodic nausea associated with food consumption, 15% had increased stool frequency and 17% had intolerance to dairy products. After 12 months,

occasional episodes of regurgitation were still reported by 25% and a small number of patients (n= 4) reported nausea and diarrhoea (Ludwig et al. 2001). In other studies assessing patients after oesophagectomy, between 10% and 50% of patients complained of diarrhoea, nausea, reflux, bloating and abdominal pain (McLarty et al. 1997; Banki et al. 2002; De Leyn et al. 1992; Ginex et al. 2013). Similarly, in patients at least six months after gastrectomy, large-scale cross-sectional data (n= 1,153) demonstrated that abdominal pain, diarrhoea and nausea/vomiting were reported by 47%, 35% and 20% respectively (Mine et al. 2010).

Another Swedish study explored 24 patients' QoL and symptoms preoperatively and at three and 12 months following upper-GI surgery for malignancy (tumour site not described) (Olsson et al. 2007). With respect to GI symptoms, there was a significant worsening of reflux symptoms between the three- and 12 month time points ($p= 0.008$). Patients' experiences of abdominal pain were approximately the same both pre- and postoperatively. Indigestion was reported as the most discomforting of all the symptoms recorded and it had not improved by 12 months. Diarrhoea increased in the period up to three months ($p< 0.01$) and at 12 months was nearly at the same level as before surgery. There were no significant differences noted in constipation symptoms between the pre- and postoperative periods. Overall, this study demonstrated that patients at 12 months had the same GI symptoms as they had before surgery, although some were more severe (Olsson et al. 2007).

Symptoms related to acid or biliopancreatic reflux have also been described in OG cancer patients, particularly surgical patients. Commonly reported symptoms include nausea, heartburn, regurgitation, dysphagia, odynophagia and vomiting (Lerut & van Lanschot 2004). Up to 72% and 26% of patients report reflux symptoms after oesophagectomy and gastrectomy respectively (Shibuya et al. 2003; Fukuhara et al. 2002; Gutschow et al. 2001). In their retrospective study of 80 patients post-oesophagectomy, De Leyn et al. reported that 15% of patients had heartburn and regurgitation at three months and this increased to 21% after 12 months (De Leyn et al. 1992). Gutschow et al. measured reflux symptoms in 91 subtotal

oesophagectomy patients at three years or more after surgery and found that 36% of patients reported reflux in the remnant oesophagus at this time (Gutschow et al. 2001).

Qualitative interviews also provide insight into the impact that GI symptoms have on patients. A study of 17 post-oesophagectomy patients participating in focus-groups identified a theme of *'losing control of elimination'* from the patient interviews (Malmström et al. 2013). Diarrhoea and constipation were symptoms described as difficult. Problems with excessive gas and borborygmi were also described, which were said to affect their everyday lives. Interestingly, neither of these symptoms is captured in the GI symptom tools and QoL symptom scales used in the quantitative studies previously discussed.

Table 1-2 summaries the data from the key studies reported in this section with regard to the prevalence of GI symptoms in patients following surgery for OG cancer. Only studies including surgical patients were added, as there are few symptom data available for those receiving chemoradiation alone. As demonstrated by the table, data are not available for many studies for both the acute and chronic postoperative periods. Also, there are a limited number of GI symptoms included in some studies and there are no data on symptoms of belching, abdominal gurgling, flatulence, faecal urgency, incomplete evacuation and faecal incontinence. As such, it is likely that GI symptoms important to patients have been underestimated in the literature. In addition, only one study included a preoperative time point to assess the trajectory of symptoms following surgery, allowing the determination of the cause for new-onset symptoms. It is apparent that there is a need for a comprehensive assessment of all GI symptoms relevant to OG cancer at the following time points: before the commencement of treatment, during treatment and after completion of treatment.

Table 1-2 Key studies reporting the prevalence of gastrointestinal symptoms in patients following surgery for oesophagogastric cancer

		Symptom prevalence (%) where available			
Symptom	**Surgery type**	**3 months postop**	**12 months postop**	**Other postop time point**	**Reference**
Dysphagia	Oesophagectomy	-	-	6 months: high mean scores	Viklund et al. 2006
	Oesophagectomy	-	38%	2 weeks: 52%	Ludwig et al. 2001
	Oesophagectomy	27%	15%	-	De Leyn et al. 1992
	Oesophagectomy	-	22%	6 months: 30%	Ginex et al. 2013
Odynophagia	Oesophagectomy	-	-	6 months: high mean scores	Viklund et al. 2006
Acid reflux	Oesophagectomy	-	-	6 months: high mean scores	Viklund et al. 2006
	n/a	-	Significant increase in mean score from 3 months	-	Olsson et al. 2007
	Oesophagectomy	15%	21%	-	De Leyn et al. 1992
	Oesophagectomy	-	7%	12-36 months: 22%; 36+ months: 36%	Gutschow et al. 2001
	Oesophagectomy	-	-	5-217 months: 72%	Shibuya et al. 2003
	Oesophagectomy	-	45%	6 months: 37%	Ginex et al. 2013
	Gastrectomy	26%	-	-	Fukuhara et al. 2002
Food/fluid regurgitation	Oesophagectomy	-	25%	-	Ludwig et al. 2001

		Symptom prevalence (%) where available			
Symptom	**Surgery type**	**3 months postop**	**12 months postop**	**Other postop time point**	**Reference**
Nausea	Oesophagectomy	-	-	6 months: significantly more common than reference population	Viklund et al. 2006
	Oesophagectomy	-	19%	-	Ludwig et al. 2001
	n/a	-	Decrease in mean score from preop and 3 months	-	Olsson et al. 2007
	Oesophagectomy	-	26%	6 months: 30%	Ginex et al. 2013
	Gastrectomy	-	-	6-66 months: 20%	Mine et al. 2010
Abdominal discomfort/ pain	Oesophagectomy	-	35%	6 months: 37%	Ginex et al. 2013
	Gastrectomy	-	-	6 months: 10%	Visick 1948
	n/a	Same mean score as preop	Same mean score as preop	-	Olsson et al. 2007
	Gastrectomy	-	-	6-66 months: 47%	Mine et al. 2010
	Gastrectomy	37%	-	-	Fukuhara et al. 2002
Abdominal fullness/ bloating	Oesophagectomy	-	43%	6 months: 40%	Ginex et al. 2013
	Gastrectomy	-	-	6 months: 70%	Visick 1948
Vomiting	Gastrectomy	-	-	6 months: 21%	Visick 1948
	Oesophagectomy	-	-	6 months: significantly more common than reference population	Viklund et al. 2006
	n/a	-	Decrease in mean score from preop and 3 months	-	Olsson et al. 2007
	Gastrectomy	-	-	6-66 months: 20%	Mine et al. 2010

		Symptom prevalence (%) where available			
Symptom	**Surgery type**	**3 months postop**	**12 months postop**	**Other postop time point**	**Reference**
Diarrhoea	Oesophagectomy	-	8%	-	Ludwig et al. 2001
	n/a	Significant increase in mean score from baseline	Same mean score as preop	-	Olsson et al. 2007
	Gastrectomy	-	-	6-66 months: 38%	Mine et al. 2010
Increased stool frequency	Oesophagectomy	-	15%	-	Ludwig et al. 2001
Constipation	n/a	Same mean score as preop	Same mean score as preop	-	Olsson et al. 2007

Abbreviations: Postop, postoperative; Preop, preoperative; n/a, not available.
Notes: One study assessed preoperative symptoms (Olsson et al. 2007) and in another, 21% of the patients had perioperative chemotherapy and/or radiotherapy (Ludwig et al. 2001)

1.1.3.2 Causes of Gastrointestinal Dysfunction in Patients with Oesophagogastric Cancer

1.1.3.2.1 Motility Irregularities

Normal peristalsis results from an integrated interaction between neural, myogenic and hormonal control mechanisms. There is evidence to suggest that there is a high incidence of motility irregularities in patients with OG cancer even at the time of diagnosis (Tormey et al. 2003). The mechanisms for such irregularities have been studied in much greater detail in patients during treatment or following its completion rather than at the time of diagnosis.

It has been suggested that GI symptoms following surgery for OG cancer may be due to irregularities that interfere with normal processes of GI motility, gastric reservoir function or gastric emptying. The physiological causes are complex and although divergent in origin, both rapid and delayed emptying can produce remarkably similar GI symptoms: nausea, vomiting and abdominal pain tend to be the predominant symptoms in both cases, though diarrhoea is usually only seen in rapid emptying (Rostas et al. 2011).

Gastroparesis occurs when gastric (or gastric remnant, if applicable) transit is abnormally slow in the absence of a physical obstruction. It can be referred to as *'delayed emptying'* and often presents with one or more of the following symptoms: nausea, vomiting, abdominal pain, early satiety, postprandial fullness, heartburn, reflux and dysphagia. Abdominal surgery is thought to be the third most common cause of gastroparesis in clinical practice (after medications/drugs and diabetes) (Patrick & Epstein 2008). The vagus nerve normally provides extrinsic parasympathetic regulation to the stomach by modulating fundal accommodation and phasic antral peristalsis. This nerve will be cut during an antrectomy, a Roux-en-Y gastrojejunostomy or a vagotomy. The vagal denervation eliminates a critical postprandial stimulus to the enteric nervous system, leading to reduced peristalsis, a reduced ability to empty solids and a profound delayed emptying from the gastric remnant and efferent limb (Patrick & Epstein 2008; Rostas et al. 2011).

Aside from this neural disorder, it is believed that hormonal, muscular and rhythm disorders may also have a role to play in gastroparesis. With regard to hormones, cholecystokinin (CCK) and somatostatin are of interest in this context. Cholecystokinin potentiates gastric relaxation, stimulates mechanoreceptors sensitive to gastric stretch and slows gastric emptying (Luttikhold et al. 2013). Levels of CCK have been shown to be increased following gastrectomy, which is likely related to inhibition of antral motor activity and slowing of gastric emptying (Yamashita et al. 2000). Somatostatin has an anti-secretory function but also reduces intestinal motility, decreases splanchnic blood flow, and prolongs gastric emptying (Luttikhold et al. 2013). Levels of this hormone are also raised following surgery, suggesting a role in delayed gastric emptying.

The reported incidence of delayed emptying after oesophagectomy and gastrectomy ranges from 2-47% and 3-30% respectively (Kim K. H et al. 2012b; Cohen & Ottinger 1976; Jordon & Walker 1973; Bar-Natan et al. 1996; Arya et al. 2014). A systematic review of the literature with regard to the outcomes of pyloric management following oesophageal reconstruction was recently performed (Arya et al. 2014). The authors demonstrated that there was a trend favouring drainage procedures, with pyloroplasty (procedure to widen the opening in the pylorus so that gastric/gastric remnant contents can empty into the duodenum) and pyloromyotomy (procedure in which an incision is made in the longitudinal and circular muscles of the pylorus) being the most popular, which reduced the incidence of delayed emptying, although significance was not reached.

Hayami et al. were interested in measuring gastric emptying following surgery. They performed the 13C-octanoic breath test in patients after gastrectomy and examined the residual stomach's emptying ability (Hayami et al. 2011). This is an easy, non-invasive and reliable method to clinically assess the emptying function (Viramontes et al. 2001). Results showed that increased gastric emptying correlated significantly with abdominal pain, indigestion and symptom score, using the GSRS tool. The more rapid the emptying was, the worse the symptoms were. A similar trend was seen for diarrhoea, indicating a role for rapid gastric emptying in the generation of these symptoms.

In addition to the motility irregularities caused by surgery, there is some evidence to suggest that chemotherapy and radiotherapy can induce similar effects (Coia et al. 1995; Sung et al. 2012; Di Fiore & van Cutsem 2009). Following chemotherapy, delayed emptying may be responsible, in part, for anorexia, early satiety and delayed nausea and vomiting (i.e. 24 hours to five days after the start of chemotherapy) (Nelson et al. 1993; Nelson & Walsh 1993). The gastric stasis may be secondary to autonomic nervous system dysfunction, though it is likely to be multifactorial in nature (Nelson et al. 2002). It is believed that chemotherapy can induce diarrhoea by decreasing whole gut transit time, thereby reducing water absorption in the colon (i.e. osmotic diarrhoea) (Gibson & Keefe 2006; Richardson & Dobish 2007). However, transit time has not been directly measured after the administration of chemotherapy. Following radiotherapy, delayed symptoms of oesophageal injury can manifest after several months and include chronic dysphagia secondary to a motility disorder induced by muscular or nerve injury (Shadad 2013).

1.1.3.2.2 Dumping Syndrome

Dumping syndrome after surgery is the spectrum of symptoms resulting in the transit of poorly processed hyperosmolar gastric contents into the duodenum (Rostas et al. 2011). Dumping syndrome can include the following postprandial symptoms: nausea, flushing, dizziness, sweating, hypotension, abdominal cramps and diarrhoea. These symptoms can occur in two phases, (1) soon after meals (*'early dumping'*) or (2) delayed for up to several hours (*'late dumping'*).

Early dumping occurs 15 to 30 minutes after eating. When hyperosmolar diet contents swiftly enter the duodenum, this leads to a sudden osmotic fluid shift into the small bowel, resulting in nausea, vomiting, diarrhoea and hypotension (Rostas et al. 2011). Late dumping, on the other hand, typically occurs 45 to 60 minutes (but sometimes up to three hours) after eating and is likely to occur secondary to reactive hypoglycaemia. Rapid stomach (or gastric remnant, if applicable) emptying induces a high glucose (and other sugar) concentration in the small bowel lumen, followed by rapid uptake by the enterocytes causing hyperglycaemia, which stimulates

the release of insulin and leads to rebound hypoglycaemia (Lerut & van Lanschot 2004). The characteristic symptoms of late dumping syndrome include faintness, hunger, dizziness, and cold sweat (i.e. the symptoms of hypoglycaemia).

1.1.3.2.3 Surgical Technique

As discussed in Section 1.1.3.2.1, vagal denervation can reduce peristalsis and cause profound delayed emptying from the gastric remnant and (denervated) efferent limb. There are also indications that damage or removal of the vagus nerve during surgery for OG cancer alters acid secretion. It was once believed that patients following oesophagectomy had reduced acid output of the denervated stomach (used as an oesophageal substitute) (Hölscher et al. 1988). However, researchers have recently shown that the oesophageal substitute recovers a normal intraluminal acidity with time, so that more than three years after surgery, the 24-hour pH profile in the gastric cavity of almost all patients is similar to that in healthy subjects. Similarly, Hashimoto and colleagues described that gastric acidity did not decrease after oesophagectomy in 55 patients, but rather postoperative acidity in the gastric tube was high, especially in patients with high preoperative acidity (Hashimoto et al. 1995). This can result in symptoms of reflux, though there are other causes for reflux too e.g. overweight/obesity, defective cardiac sphincter muscle and hiatus hernia.

In recent years, the use of potent anti-acid medication (usually proton pump inhibitors) postoperatively appears to have reduced the prevalence of reflux symptoms in patients with OG cancer. The beneficial effect of these drugs has been demonstrated in a randomised trial involving 79 patients following oesophagectomy (Johansson et al. 2009). Those patients receiving the medication at the dose recommended for the treatment of erosive oesophagitis had significantly less gastric acid secretion postprandially ($p= 0.008$), less exposure of the remnant oesophagus to gastric acid ($p= 0.034$), a lower rate of esophagitis ($p= 0.002$), and less dysphagia ($p= 0.032$) than control patients.

Surgical technique appears to have a role in determining the development of reflux symptoms. In De Leyn et al.'s study, at 12 months following oesophagectomy, there was a statistically significant difference between patients with cervical anastomosis and those with intrathoracic anastomosis when comparing reflux symptoms (4% versus (vs.) 50%; p= 0.0001) and remnant oesophagitis (8% vs. 53%; p= 0.001) (De Leyn et al. 1992). These findings were subsequently supported by the study undertaken by Shibuya et al. In their group of 80 oesophagectomy patients, the incidence of reflux esophagitis in the cervical anastomosis group and the intrathoracic anastomosis group was 56.4% and 88.6% respectively, with a significant difference between them (p= 0.004).

For gastric cancer, the surgical reconstruction technique is also an important factor to consider. Bile reflux resulting in remnant gastritis and gastro-oesophageal reflux disease has been noted as a problem associated with Billroth I reconstruction after distal gastrectomy (Fujiwara et al. 1998). This finding was reinforced by other reports, which indicate that Roux-en-Y reconstruction following distal gastrectomy is superior to Billroth I in terms of preventing remnant gastritis and reflux oesophagitis because this procedure reduces duodenogastric and gastro-oesophageal reflux (Fukuhara et al. 2002; Ishikawa et al. 2005).

1.1.3.2.4 Gastrointestinal Hormone Abnormalities

The removal of some or the entire hormone producing mucosa of the stomach and rearranging the route for the passage of food will inevitably alter GI hormone production. Hormones that have been studied in this regard include gastrin, CCK, neurotensin, pancreatic polypeptide, serotonin and glucagon. The hormonal involvement in gastroparesis has previously been described in Section 1.1.3.2.1.

Serum concentrations of gastrin, pancreatic polypeptide, neurotensin and CCK were measured in 16 patients before and after partial gastrectomy for gastric cancer (with reconstruction by Bilroth methods) (Yamashita et al. 2000). Compared with a control group, fasting levels of CCK and neurotensin were significantly increased in the patients who had a gastrectomy. In addition,

gastrin and pancreatic polypeptide were completely abolished in these patients. The effect of these hormonal alterations is thought to result in reduced contraction of the lower oesophageal sphincter and may lead to symptoms of reflux.

Kalmar and colleagues measured plasma concentrations of insulin, CCK and somatostatin in patients with three different reconstruction types following gastrectomy (Kalmar et al. 2006). Their results supports a diabetoid blood glucose profile in the first postprandial hour in patients after gastrectomy and Roux-en-Y reconstruction, with higher insulin and CCK concentrations, and an increasing somatostatin release 15 to 30 minutes postprandially. This picture indicates that the pathophysiology of early dumping syndrome may include a hormonal imbalance. This is supported by findings that the hyperosmolar luminal contents seen in dumping syndrome cause the release of vasoactive neurotransmitters (e.g. serotonin, neurotensin, peptide YY, glucagon). Serotonin exacerbates the situation by causing peripheral and mesenteric vasodilation, adding to the hypotension-inducing fluid shifts (Rostas et al. 2011).

1.1.3.2.5 Small Intestinal Bacterial Overgrowth

Small intestinal bacterial overgrowth (SIBO) is a condition that implies abnormal bacterial colonisation of the proximal small bowel (Gasbarrini et al. 2007; Rana & Bhardwaj 2008; Dukowicz et al. 2007). In health, the acidity of the stomach and the relatively swift peristalsis through the stomach and small bowel means that the stomach, duodenum and jejunum contain only low levels of microbiota, which range from 10^3 to 10^5 colony-forming units per millilitre (CFU/ml) of luminal contents. This is made up mainly of acid-resistant Lactobacilli and Streptococci (Hao & Lee 2004). The duodenum and jejunum act as a transition zone leading to the ileum where the microbiota begin to resemble those of the colon, with around 10^7-10^8 CFU/ml. The colon is the primary site of microbial colonisation in humans with approximately 10^{11}-10^{12} CFU/ml of contents (Power et al. 2013). The main types of colonic microbiota are obligate anaerobes, with the most abundant being members of the genus Bacteroides, anaerobic Gram-positive cocci such as Peptostreptococcus species (sp.), Eubacterium sp., and Clostridium sp.

Small intestinal bacterial overgrowth results from the failure of intrinsic defence mechanisms that restrict bacterial proliferation. Under normal physiological conditions, low microbial numbers in the small bowel is maintained by the following mechanisms: normal interdigestive migrating motor complex activities propel intraluminal contents towards the colon; actions of gastric, biliary and pancreatic enzymes limit bacterial growth; the mucosa's protective mucous layer traps bacteria; and the ileo-caecal valve inhibits retrograde translocation of bacteria from the colon into the ileum (Trespi & Ferrieri 1999; Lombardo et al. 2010; Deloose et al. 2012; Miller et al. 2012; George et al. 2012; Rana et al. 2011; Faria et al. 2013). The pathophysiology of SIBO is pictorially described in Figure 1-1.

There is no agreed definition for SIBO, but the most commonly cited definition is quantitative: 10^5 or more CFU/ml of bacteria grown from a small bowel aspirate collected during an oesophago-gastroduodenoscopy (OGD) (Khoshini et al. 2008). The validity of this cut-off level has not been confirmed, despite its widespread use (Khoshini et al. 2008). Similarly, there is no gold-standard test for its diagnosis. The ongoing debate over its diagnosis will be discussed in Section 1.3.1.

Patients with SIBO may be clinically asymptomatic or have symptoms that fulfill the diagnostic criteria of irritable bowel syndrome (IBS): Rome III criteria define IBS as pain associated with change in bowel habit (Drossman 2006). In addition, research in IBS suggests that bloating, diarrhoea and flatulence are important symptoms, which are believed to be common in SIBO too (Yamini & Pimentel 2010; Pimentel et al. 2000; Reddymasu et al. 2010). However, there are few studies, which have focused on identifying the predominant clinical symptoms in patients with SIBO. Those that have, suggest that the most common GI symptom caused by SIBO is diarrhoea, followed by abdominal pain, and then bloating (Grace et al. 2013). Other troublesome symptoms reported in the literature include flatulence, abdominal tenderness, constipation, gastric stasis, steatorrhoea, increased stool frequency, nausea and weight loss (Lombardo et al. 2010; Tursi et al. 2003; Marie et al. 2009; Klaus et al. 2009; Compare et al. 2010; Pimentel et al. 2000; Roberts et al. 1977; Davidson et al. 1984; Choung et al. 2012).

Classically, the recognised causes for SIBO were blind intestinal loops, which occur when part of the bowel is by-passed (Hamilton et al. 1970). The five types of blind loop include those caused by (a) anastomosis with formation of self-filling loop, (b) jejunal diverticulosis, (c) intestinal stricture, (d) enterostomy or fistula, and (e) gastric surgery. In fact, SIBO was once known as the *'blind loop syndrome'* or the *'stagnant loop syndrome'*. In recent years, there has been renewed interest in SIBO as it has been implicated in the pathophysiology of conditions previously not classically associated with it. These clinical conditions and their reported prevalence rates are shown in Table 1-3. This list is not exhaustive.

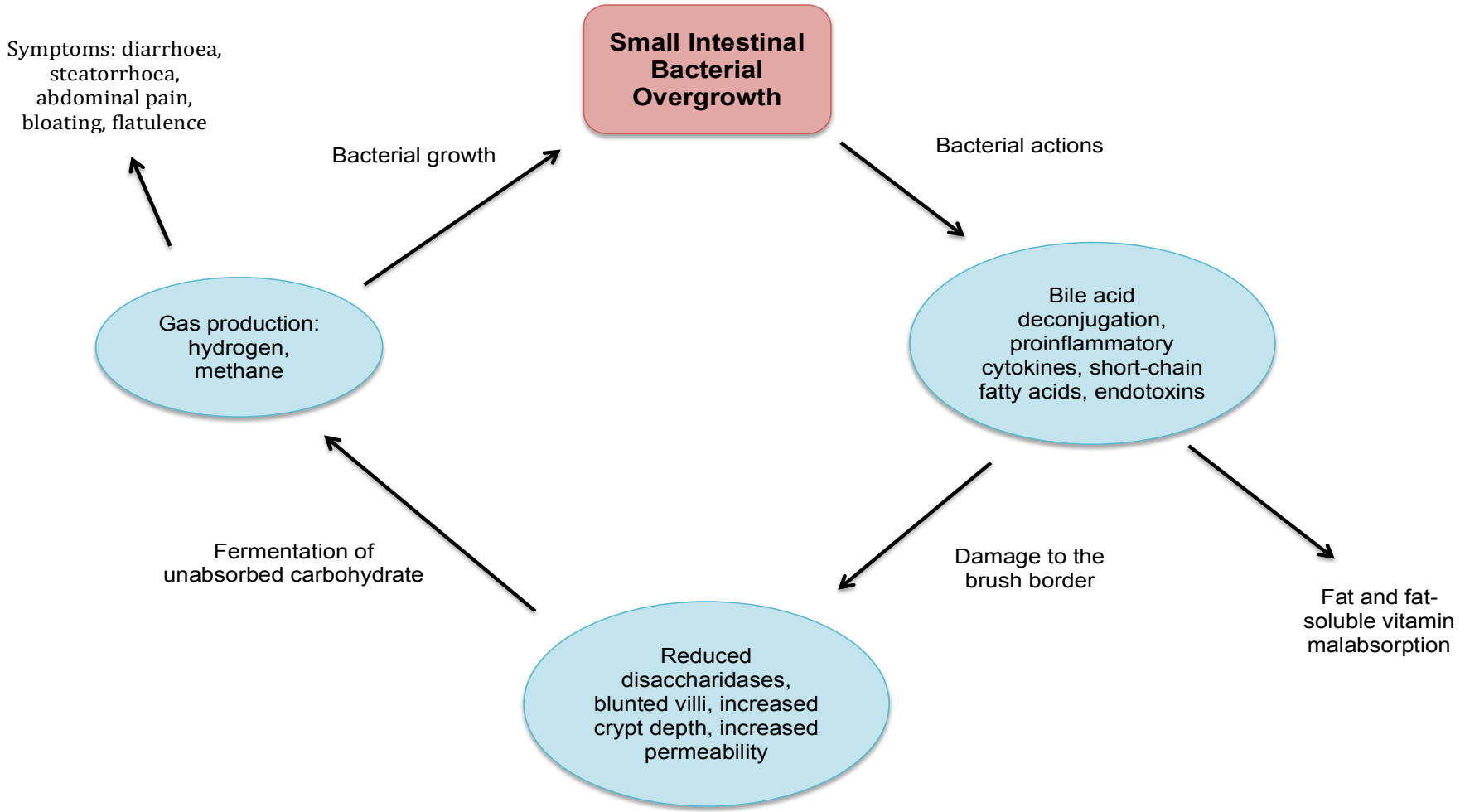


Figure 1-1 Pathophysiology of small intestinal bacterial overgrowth

Table 1-3 Reported prevalence of small intestinal bacterial overgrowth in normal populations and disease states

	Reported prevalence	References
Healthy study controls	0-20%	Lombardo et al. 2010; Sabaté et al. 2008; Posserud et al. 2007; Teo et al. 2004; Lewis et al. 1999; Pimentel et al. 2003; Rana et al. 2011; Bratten et al. 2008; Scarpellini et al. 2009
Dysmotility/Gut wall injury		
Coeliac disease	9-67%	Rana et al. 2007; Rubio-Tapia et al. 2009; Tursi et al. 2003
Connective tissue diseases	43-55%	Marie et al. 2009; Parodi, Sessarego, et al. 2008b
Diabetes mellitus	8-44%	Rana et al. 2011; Lin & Pimentel 2012
Hypothyroidism	54%	Lauritano et al. 2007
Inflammatory bowel disease	25-88%	Funayama et al. 1999; Klaus et al. 2009; Lin & Pimentel 2012; Ibanez et al. 2008
Nonspecific dysmotility	76%	Jacobs et al. 2013
Radiation enteropathy	26%	Wedlake et al. 2008
Neuromuscular Diseases		
Muscular dystrophy	65%	Tarnopolsky et al. 2010
Parkinson's disease	54%	Gabrielli et al. 2011
Surgery		
Abdominal Surgery	82%	Petrone et al. 2011
Bilateral truncal vagotomy	93%	Browning et al. 1974
Gastrectomy	37-100%	Brägelmann et al. 1997; Paik et al. 2011; Bjorneklett et al. 1983; Iivonen et al. 1998; Lock et al. 1995
Ileo-caecal valve resection	32%	Klaus et al. 2009
Roux-en-Y reconstruction	86%	Iivonen et al. 1998

	Reported prevalence	References
Miscellaneous		
Chronic fatigue syndrome	81%	Lin & Pimentel 2012
Chronic pancreatitis	34-92%	Trespi & Ferrieri 1999; Mancilla et al. 2008
Drug-induced acid suppression	26-75%	Lombardo et al. 2010; Jacobs et al. 2013; Compare et al. 2010
End-stage renal failure	36%	Strid et al. 2003
Fibromyalgia	93%	Lin & Pimentel 2012
Irritable bowel syndrome	4-78%	Posserud et al. 2007; Bratten et al. 2008; Pimentel et al. 2000; Nucera et al. 2005; Lupascu et al. 2012; Grover et al. 2008
Immunodeficiency syndromes	30-50%	Smith et al. 1990
Liver cirrhosis	17-36%	Yang et al. 1998; Gunnarsdottir 2003
Obesity	17-41%	Sabaté et al. 2008; Madrid et al. 2011
Parenteral nutrition	70%	Gutierrez et al. 2012
Rosacea	46%	Parodi, Paolino, et al. 2008a

There are published data indicating that oncology patients may be at increased risk of developing SIBO. For example, there are case reports in pancreatic cancer, ovarian cancer, small bowel lymphoma and small bowel leiomyosarcoma (Bustillo et al. 2009; Russell et al. 1977; Takagi et al. 2002; Swan 1974), as well as small hypothesis-generating studies in chronic lymphocytic leukaemia patients and a mixed cohort of GI, gynaecological and urological cancer patients (Wedlake et al. 2008; Smith et al. 1990).

Also, clinical experience at The Royal Marsden (RM) informs us that SIBO is a condition of relevance in patients attending gastroenterology clinics for new onset GI symptoms related to their cancer treatment (any primary diagnosis). A retrospective review of the records of 435 patients referred for investigation of GI symptoms potentially caused by SIBO was performed (unpublished data). Each patient underwent at least one of two commonly used diagnostic tests: the glucose hydrogen methane breath test (GHMBT) and endoscopic aspiration and culture technique (test details in Section 2.4). Of those included, 49 (11%) patients had been treated for a primary cancer occurring in the upper-GI tract. In total, 46 patients (10.6%) had a diagnosis of Definite SIBO, 74 patients (17.1%) had Possible SIBO, 115 (26.4%) did not have SIBO and 200 (45.9%) were not assessable due to missing data. Of those with Definite- or Possible SIBO, almost a quarter had an upper-GI cancer diagnosis, compared with 1% in the No SIBO group. The retrospective nature of this study means that there is a limit to how far the data can be reliably interrogated. In addition, not every patient had a complete GHMBT or available aspirate culture results and some were not followed-up. In total, this represented 30% of the study population and may have resulted in some missed cases of SIBO.

Another study undertaken at RM suggests that SIBO is a condition of importance in the oncology setting. In this study, 38 consecutive patients with GI, gynaecological and urological cancers were recruited (Wedlake et al. 2008). All patients were planned for radical or adjuvant pelvic radiotherapy. To define the development of new SIBO, glucose-H_2 breath tests were performed at baseline and at four-five weeks after the commencement of treatment, irrespective of the presence of GI symptoms. Ten patients (26%) with a negative test at baseline, tested

positive for SIBO at four-five weeks. Of these patients, two (20%) had normal bowel movements at baseline and at follow-up and six (60%) developed new-onset increase in bowel frequency or a change in the quality of bowel habit. The remaining two patients had existing bowel problems at baseline, which either remained stable or improved. The authors suggested that pelvic radiotherapy was the cause for SIBO in this cohort (Wedlake et al. 2008; Swan 1974). Other data from patients with chronic GI symptoms following gynaecological cancer indicates that radiotherapy was the main cause for SIBO (Husebye et al. 1994). The potential mechanism for its development following radiotherapy may be multifactorial, with previous intestinal resections, intestinal strictures, epithelial dysfunction, intestinal dysmotility and hypochlorhydria potentially involved.

Chemotherapy can cause extensive damage to the GI tract, leading to functional and structural changes (Cunningham et al. 1985). In fact, histological evidence of damage can be seen in the jejunum as early as 30 minutes after treatment, with greatest damage seen 24 hours later (Stringer, Gibson, Logan, et al. 2009a). Some recent studies have implicated the intestinal microbiota, though not specifically SIBO, in the adverse side-effects of chemotherapy, but this is a relatively new area of research and very little exists in the literature (Stringer, Gibson, Bowen, et al. 2009b).

Stringer et al. undertook a study in rats investigating changes in mucin secretion and microbiota following treatment with 5-fluorouracil chemotherapy (Stringer, Gibson, Logan, et al. 2009a). Mucins are large glycoproteins produced by goblet cells, which provide attachment sites for resident bacteria and pathogenic bacteria, as well as simultaneously protecting the mucosa from SIBO and/or penetration (Robbe et al. 2004; Specian & Oliver 1991). Results from Stringer's study clearly demonstrated changes in the microbiota of the jejunum. There were decreases in Clostridium sp. and Lactobacillus sp., with an increase in Escherichia sp. 48-72 hours after treatment. Organisms involved in maintaining balance in the intestine were susceptible to 5-fluorouracil, while *Pseudomonas aeruginosa* and *Escherichia coli* were not. This may allow the proliferation of these non-susceptible opportunistic bacteria, potentially

causing SIBO. In addition, goblet cells in the jejunum decreased after treatment with the chemotherapy, with crypt goblet cells being affected the most, decreasing significantly from 24-72 hours after treatment. Alterations in the protective capacity of the mucus barrier could also lead to the development of SIBO (Stringer, Gibson, Logan, et al. 2009a). There are some other animal studies that have implicated altered microbiota or goblet cell functioning as the causes for chemotherapy induced side-effects (Stringer, Gibson, Bowen, et al. 2009b; Verburg et al. 2000). However, further studies are warranted to fully elucidate these relationships, particularly with regard to the pathophysiology of SIBO.

To date, the greatest body of evidence linking oncological treatments to SIBO exists for surgical procedures. The creation of self-filling blind loops in animal experiments were frequently used in the 1970's and 1980's in order to investigate the pathophysiology of SIBO (Sherman & Lichtman 1987; Schjönsby & Hofstad 1972; Giannella & Toskes 1976). Today, this experimental model is still relevant as the structural conditions necessary for the blind loop are present after Roux-en-Y gastric by-pass and gastrectomy with Roux-en-Y reconstruction (Di Stefano et al. 2005).

Data regarding the frequency of SIBO after gastric surgery strongly suggests an association. For example, Lock et al. found SIBO in 35 of 38 (92%) patients after gastric surgery of varying types (1995). However, this study recruited patients referred for a gastroscopy and so a selection bias needs to be considered. Another group studied 22 patients after a Billroth II resection, all of whom had SIBO (Bjorneklett et al. 1983). Larger studies also reported high prevalence rates e.g. in a study of 127 patients after total gastrectomy, SIBO was diagnosed in 37% (Brägelmann et al. 1997). Another study of 46 total gastrectomy patients found that most (88%) had SIBO (Iivonen et al. 1998). While Paik et al. reported that 78% of their 76 gastrectomy patients had SIBO, which appeared to be the cause for their postprandial GI symptoms (Paik et al. 2011). In addition, in a study of older patients (n= 168) with symptoms suggestive of SIBO, previous partial gastrectomy was shown to be one of the characteristics

predictive of SIBO ($p < 0.01$) (Elphick et al. 2005). There does not appear to be any data in the literature on SIBO prevalence following oesophagectomy.

The underlying mechanism for SIBO development is likely to be multifactorial including both anatomical and functional conditions as shown in Table 1-4. Achlorhydria and impaired motility, are likely to be the primary factors responsible for controlling the number of bacteria in the small bowel (Henderson & Wilson 1996). In more severe cases of SIBO, there may be signs of malabsorption (e.g. weight loss and steatorrhoea). The mechanisms causing malabsorption of different macro- and micronutrients in SIBO are outlined in Table 1-5.

Table 1-4 Mechanisms for the development of small intestinal bacterial overgrowth (SIBO) in oesophagogastric cancer

Mechanism	Description of mechanism	Reference (s)
Dysmotility	Cleansing action of propulsive forces, and especially, phase III of the interdigestive migratory motor complex, limits the ability of the bacteria to colonise the small bowel by sweeping its contents towards the colon. Lack of phase III predisposes to SIBO. Delayed orocaecal transit time has been shown to be associated with the presence of SIBO.	Tomita et al. 2006; Vantrappen et al. 1977; George et al. 2012; Kaur et al. 2014
Hypochlor-hydria	The use of proton pump inhibitors causes suppression of gastric acid secretion, which increases the gastric pH and facilitates the survival and colonisation of bacteria including viable swallowed bacteria. Hypochlorhydria has been shown to contribute to proximal migration of more distally located bacteria in the gastrointestinal tract.	Jacobs et al. 2013; Lombardo et al. 2010; Compare et al. 2010
Enterocyte dysfunction	Villus and crypt hypertrophy and a decreased villus to crypt ratio have been seen in experimental models of SIBO. Bacterial production of short-chain fatty acids and a decreased intraluminal pH can lead to direct or indirect damage to enterocytes. Mucosal breaks (erosions and ulcerations) have been described in SIBO. SIBO may also induce an inflammatory response in the intestinal mucosa. It may result in microscopic mucosal inflammation and has been associated with increased serum endotoxin and bacterial compounds stimulating production of pro-inflammatory cytokines. Pro-inflammatory cytokines (interleukin (IL)-6, IL-8 and tumour necrosis factor alpha), and lipid peroxidation (marker of oxidative stress) have been shown to be significantly higher in the plasma of patients with SIBO compared with those without it.	Toskes et al. 1975; Gilroy et al. 2003; Hoog et al. 2007; Saltzman et al. 1994; Wanitschke & Ammon 1978; Saltzman & Russell 1994
Altered anatomy	A change to the normal anatomy of the GI tract may cause stagnation of intraluminal flow and cause bacterial stasis in the small bowel. It may also lead to abnormal motility and ineffective clearance of retained food and secretions.	Brägelmann et al. 1997; Iivonen et al. 1998
Altered intestinal mucus layer	The integrity of the normal intestinal microbiota is maintained by the structure of mucins. In SIBO there is a significant increase in luminal mucins and a fall in mucosal mucins. The luminal mucins should act to protect the mucosa from SIBO, but in fact, they do not completely preserve mucosal enterocyte structure and function. Anaerobic bacteria produce proteases that are capable of destroying brush border disaccharidase activity.	Stringer, Gibson, Logan, et al. 2009a; Sherman et al. 1987
Ileo-caecal valve dysfunction	A functioning valve inhibits retrograde translocation of bacteria from the colon into the ileum. Individuals with SIBO are more likely to have a defective ileo-caecal valve (with low ileo-caecal junction pressures) than individuals without SIBO.	Miller et al. 2012; Roland et al. 2014
Altered immune function	Individuals with SIBO are more likely to have abnormalities in intestinal mucosal immunity as evidenced by increased luminal immunoglobulin A concentrations and lamina propria immunoglobulin A plasma cell counts.	Riordan et al. 1999; Riordan, McIver, Wakefield, et al. 1997b
Pancreatic insufficiency	Pancreatic enzymes have an important influence on small bowel microbiota. Proteolytic enzymes have anti-bacterial properties when they enter the duodenum. If enzyme levels are reduced, bacteria can proliferate.	Pezzilli 2009

Table 1-5 Mechanisms for macronutrient and micronutrient malabsorption in small intestinal bacterial overgrowth (SIBO)

Nutrient	Description of malabsorption	Reference(s)
Fat	Fat malabsorption may result from SIBO and is principally due to bacterial deconjugation of bile acids and subsequent deficiency of intraluminal conjugated bile acids. In health, water-soluble conjugated bile salts are secreted to form mixed micelles consisting of partially digested dietary lipids. Such bile salts are not readily reabsorbed until they reach the ileum. In SIBO, the microbiota deconjugate bile acids resulting in free bile acids, which are readily absorbed by the jejunum. This process can lead to impaired formation of the bile-salt-lipid micelle complex and resultant dietary fat malabsorption. Free bile acids formed may also be toxic to the mucosa.	Kim et al. 1966; Singh & Toskes 2004; Wanitschke & Ammon 1978
Carbohydrate	The presence of a blind loop causes a progressive loss of brush border disaccharidase function beginning with the loss of lactase activity. The exact reason for the disaccharidase deficiencies remains unknown. Small bowel bacteria can ferment the malabsorbed carbohydrates to form short-chain fatty acids, leading to concentrations in jejunal aspirates four times higher than those in healthy subjects. These short-chain fatty acids can cause direct damage to enterocytes, which leads to further loss of activity of brush border disaccharidases.	Sherman et al. 1985; Hoverstad et al. 1985
Protein	There are numerous factors that can contribute to protein malabsorption in SIBO. The bacteria may be responsible for deaminating dietary protein in the gut lumen. Thus, there is a diversion of dietary nitrogen into urea formation and it becomes unavailable for protein anabolism by the human host. Also, direct or indirect damage to enterocytes can lead to altered permeability, which allows for protein leakage from the blood into the gut lumen (protein-losing enteropathy). If the enterocytes are damaged, amino acids absorption is impaired.	Jones et al. 1968; King & Toskes 1981; Giannella et al. 1974; MacMahon et al. 1994
Fat-soluble vitamins	Malabsorption of vitamins A, D or E may occur in association with general fat malabsorption and is caused by similar mechanisms. In vitamin D deficiency, hypocalcaemia, osteomalacia or osteoporosis can be complications of SIBO. In severe cases, hypocalcaemia tetany has been reported. There are only case reports of deficiencies of vitamins A and E. Levels of vitamin K are usually normal or raised due to bacterial synthesis of menaquinone.	Stotzer et al. 2003; Saltzman et al. 1994; Kowdley et al. 1992
Vitamin B_{12}	Megaloblastic, macrocytic anaemia can occur in SIBO due to a vitamin B_{12} deficiency. In the duodenum and jejunum, vitamin B_{12} binds to intrinsic factor enabling its absorption in the distal ileum. However, in SIBO, Gram-negative bacteria are capable of competitively utilising vitamin B_{12} i.e. bacteria use the vitamin to produce inactive cobamides, which compete with dietary vitamin B_{12} for ileal binding sites. Paradoxically, the cobalamin synthesised by bacteria is retained by them, and thus it remains mostly unavailable for host absorption.	Saltzman & R. Russell 1994; Welkos et al. 1981; Giannella et al. 1972
Folic acid	Bacteria in the small bowel may utilise folic acid. Ironically these bacteria also produce the vitamin, which is available to the host. Therefore, serum folate concentrations are typically normal or even elevated in SIBO.	Russell et al. 1986
Iron	Iron deficiency anaemia can occur, though the exact mechanism is unknown: it is likely that iron deficiency is due to abnormal intestinal conditions (e.g. a blind loop), where ulceration leads to blood loss causing detectable faecal occult blood. Damage to the mucosa by bacterial toxins, short-chain fatty acids or unconjugated bile acids may inhibit iron absorption. A mixed anaemia will result if the patient has both iron and vitamin B_{12} deficiencies.	Giannella & Toskes 1976; Iivonen et al. 1998

Weight loss has been described as a feature of SIBO. In a study of 150 patients with Crohn's disease, those who tested positive for SIBO showed a significantly lower body weight than did patients without SIBO (63.6 kg vs. 70.4 kg, p= 0.014) (Klaus et al. 2009). Haboubi and Montgomery described 16 elderly patients with SIBO (1992). Following treatment for it, 13 gained weight: there were highly significant increases in body weight, mid-arm muscle circumference and triceps skin-fold thickness. Similarly, in a larger study of 168 symptomatic elderly patients, those with SIBO were more likely to have lost weight than those without SIBO (44.9% vs. 27.7%, p= 0.05) (Elphick et al. 2005). The period over which weight was lost was not reported. There has been one study, which suggests that the weight loss seen in OG cancer may be associated with SIBO. Paik et al.'s study of patients following total gastrectomy (n= 78) found there to be a negative correlation between the presence of SIBO and postoperative weight loss (2011). This suggests the importance of SIBO in the postoperative nutrition of OG cancer patients.

The primary goal of therapy in SIBO should be the treatment of any underlying disease or structural defect, although for many conditions this cannot be achieved. The management should include the correction of any nutritional deficiencies, where present. The cornerstone of treatment of SIBO is the use of antibiotics to modify the GI microbiota and improve symptoms. Effective treatment generally involves one or more antibiotic regimens with activity against aerobic and anaerobic organisms. Many different regimens have been advocated, including ciprofloxacin, metronidazole, neomycin, norfloxacin and doxycycline. There is no consensus on the most efficacious dose of antibiotics or duration of treatment. Recently, there has been a growing interest in the use of rifaximin (a non-absorbable rifamycin analogue) in the treatment of SIBO, especially in patients with IBS (Di Stefano et al. 2005; Lauritano et al. 2005; Chang & Green 2012; Pimentel et al. 2006). It has been shown to be efficacious and has good short-term safety in this setting (Menees et al. 2012; Pimentel et al. 2014; Zhao et al. 2014), but similar studies have not been undertaken specifically in SIBO.

Recently, a systematic review and meta-analysis was performed that compared the clinical effectiveness of antibiotics in the treatment of symptomatic IBS patients with documented SIBO (Shah et al. 2010). Of the ten studies that met the inclusion criteria, rifaximin was the most studied antibiotic (eight studies). Meta-analysis of four studies favoured antibiotics over placebo for successful treatment of SIBO with an odds ratio (OR) of 2.55 (95% confidence interval (CI) 1.29–5.04). However, trials involving larger patient populations, comparing a greater diversity of antibiotics with one another and with placebo are needed to determine an optimal treatment strategy. Importantly, large prospective studies of SIBO in patients with cancer have not been undertaken, despite the SIBO-like GI symptoms that they experience and the risk factors that they may have.

1.1.4 Nutritional Status in Patients with Oesophagogastric Cancer

1.1.4.1 Defining Malnutrition in Oncology

Malnutrition can be defined as a '*state of nutrition in which a deficiency, excess or imbalance of energy, protein, and other nutrients causes measurable effects on tissue (shape, size, composition), function and clinical outcomes*' (Stratton et al. 2003). Although, this term encompasses overnutrition, for the purpose of this thesis the term *malnutrition* shall be synonymous with *undernutrition*. The cause of malnutrition in cancer is multifactorial in nature, resulting from inadequate dietary intake, increased requirements, impaired nutrient absorption and transport and/or altered nutrient utilisation, as well as, the effect of antineoplastic treatments (White et al. 2012).

Until recently, there has been no single, universally accepted approach to the diagnosis and documentation of adult malnutrition. This may explain a survey in the UK demonstrating that 80% of specialist oncological trainees expressed uncertainty or a lack of confidence in their ability to identify malnutrition (Spiro et al. 2006). This is a widespread problem with a similar study in the US demonstrating that 88% of radiology oncologists measured only body weight in order to determine malnutrition, while just 9% of them used body weight plus other assessment approaches (DeCicco et al. 2010). As such, despite the recommendations that standardised

and validated nutrition assessment approaches be used for patients with cancer, nutritional status is often not systematically evaluated in clinical practice (Elia 2003; Kondrup, Allison, et al. 2003b).

In 2009, an international guideline committee of nutrition experts from the American Society for Parenteral and Enteral Nutrition (ASPEN) and the European Society for Clinical Nutrition and Metabolism (ESPEN) was convened. Their aim was to develop a consensus approach to defining malnutrition for adults in the clinical setting. The committee produced three aetiology-based definitions for diagnosing malnutrition, which highlight (a) the depletion of body cell mass due to reduced intake or assimilation of energy/protein and (b) the inflammation which promotes catabolism of skeletal muscle (Jensen et al. 2010). With regard to the latter, the contribution of inflammation to malnutrition is important to consider as nutrient requirements are altered by the inflammatory response (Zoico & Roubenoff 2002). If the catabolism of muscle tissue results in skeletal muscle mass greater than two standard deviations (SD) below that typical of healthy adults, the individual has sarcopenia (Baumgartner et al. 1998). The three aetiology-based definitions proposed by the committee are as follows:

- **Starvation-related malnutrition** which is pure chronic starvation (e.g. anorexia nervosa)
- **Chronic disease-related malnutrition** related to chronic inflammation of a mild-moderate degree (e.g. organ failure, pancreatic cancer, rheumatoid arthritis)
- **Acute disease or injury-related malnutrition** related to acute inflammation of a severe degree (e.g. major infections, burns, trauma or closed head injury)

The malnutrition experienced by patients with cancer is most likely to be chronic disease-related malnutrition, whereby they experience a chronic weight loss associated with an imbalance between pro- and anti-inflammatory cytokines. Though, cancer patients may also experience acute disease or injury-related malnutrition following an acute episode e.g. following surgery or a serious infection. Once these aetiology-based definitions were agreed upon, the Academy of Nutrition and Dietetics and ASPEN developed a list of metrics to detect and diagnose malnutrition (White et al. 2012). The group agreed that there was no single parameter that was

definitive for diagnosing adult malnutrition. The identification of two or more of six characteristics was recommended for diagnosis of malnutrition (Table 1-6). Criteria are available that delineate how to measure each of the characteristics for the accurate diagnosis of malnutrition (Appendix 8.2) (White et al. 2012). At present, there is insufficient evidence to allow for distinction to be made between mild and moderate forms of malnutrition using these characteristics. Of note, the National Institute for Health and Care Excellence describe a different definition for malnutrition compared with the Academy of Nutrition and Dietetics and ASPEN as follows: the presence of (a) a body mass index (BMI) of less than 18.5 kg/m^2, (b) unintentional weight loss greater than 10% within the last three-six months, or (c) a BMI of less than 20 kg/m^2 and unintentional weight loss greater than 5% within the last three-six months (National Institute for Health and Care Excellence 2006).

Some malnourished patients may develop cachexia. The term cachexia originates from the Greek words kakos (*'bad'*) and hexis (*'condition'*). Cachexia may develop in different diseases, for example cancer, congestive heart failure and human immunodeficiency virus infection/acquired immunodeficiency syndrome. For simplicity purposes it describes severe wasting from any cause including starvation and disease (Lochs et al. 2006).

Over the past decade, our understanding of cancer cachexia has progressed but until recently there lacked a definition for it and there were no diagnostic criteria nor classification system for it. However, in 2011, an international consensus panel (with members from Europe, Canada and the US) developed a definition, diagnostic criteria and a classification system specific to cancer cachexia (Fearon et al. 2011). They defined it as '*a multifactorial syndrome characterised by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment*' (Fearon et al. 2011).

Cancer cachexia is a spectrum with three stages of clinical relevance: pre-cachexia, cachexia, and refractory cachexia. Not all patients traverse the entire spectrum and this will depend on the

cancer type and stage of disease, the presence of systemic inflammation, low food intake, and lack of response to anticancer therapy. Refractory cachexia is associated with active catabolism, or the presence of factors that render active management of weight-loss no longer possible or appropriate (Fearon et al. 2011). The diagnostic criteria developed by the panel for patients who have not entered the refractory period are shown in Table 1-6 (Fearon et al. 2011). Malnutrition (in the context of cancer) and cancer cachexia are not synonymous with one another. There are many features of malnutrition that are also features of cancer cachexia. However, other features are believed to be unique to cancer cachexia and as such it is considered to be a more multidimensional and complex syndrome (Fearon et al. 2011). Therefore, while not all malnourished patients are cachectic, all cachectic patients are invariably malnourished (Muscaritoli et al. 2010). The content of Table 1-6 facilitates discrimination between the two terms.

The features of malnutrition in cancer and cancer cachexia are described in detail in Table 1-7. Given that there is no validated tool to identify when a malnourished patient is also cachectic, most studies do not distinguish between the two entities. As such, when malnutrition prevalence data are reported, within the group deemed to be malnourished, there will inevitably be a sub-group of cachectic patients, though these data will rarely be reported. Therefore, for the purpose of this thesis, the term *malnutrition* will encompass *malnutrition in cancer* and *cancer cachexia*.

Table 1-6 Discrimination between malnutrition in cancer and cancer cachexia

	Malnutrition in cancer	Cancer cachexia
Definition	State of nutrition in which a deficiency of energy, protein, and other nutrients causes measurable effects on tissue (shape, size, composition), function and clinical outcomes	A multifactorial syndrome characterised by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment
Diagnostic Criteria (International consensus)	Any 2 or more of 6 features (a) Insufficient energy intake (b) Weight loss (c) Loss of muscle mass (d) Loss of subcutaneous fat (e) Localised or generalised fluid accumulation that may sometimes mask weight loss (f) Diminished functional status as measured by hand-grip strength	Any 1 of 3 features (a) Weight loss > 5% over the past six months (in the absence of simple starvation) (b) A BMI < 20 kg/m^2 with weight loss > 2% (c) Appendicular skeletal muscle index consistent with sarcopenia (males < 7.26 kg/m^2; females < 5.45 kg/m^2) and any degree of weight loss
Validated tools to identify the condition	Subjective Global Assessment and Patient Generated Subjective Global Assessment	None. A comprehensive, yet simple, framework for the clinical assessment of patients is an aim of the international consensus panel.
Common features	Anorexia Reduced availability of nutrients Weight loss Loss of muscle mass and strength Loss of fat mass Fluid accumulation Increased metabolism Inflammatory factors Catabolic factors Negative functional effects Psychosocial effects	
Treatment	Nutritional support as a unimodal therapy is likely to be effective	As the majority of cachectic patients probably have more than one component to their cachexia, nutrition as a unimodal therapy is unlikely to be effective in reversing cachexia. Other therapies if indicated are: physical activity prescription, anti-inflammatory drugs or nutrients, other drugs e.g. appetite stimulants, substrate normalisation approaches and psychotherapeutic therapy.
Abbreviations: BMI, body mass index References: Jensen et al. 2010; Aapro et al. 2014; Fearon et al. 2011; Muscaritoli et al. 2010		

Table 1-7 Features of malnutrition in cancer and cancer cachexia

Component	Description	Research Findings	Effect
Anorexia, reduced availability of nutrients and weight loss	Loss of desire to eat in cancer can be produced by the tumour independently of that produced by treatment.	Anorexia has been related to weight loss in the majority (40%), but not in all malnourished cancer patients (Blum et al. 2011). Energy intake relates unreliably to weight loss in studies where prospectively collected dietary assessment methods are used (Blum et al. 2011). Impaired absorption, altered transport, and altered nutrient utilisation can reduce the availability of nutrients (White et al. 2012).	Decreased food intake alone cannot explain the progressive weight loss seen in cancer (Heber et al. 1992). Malnutrition and cachexia appear to be explained by uncoupling of food intake to energy expenditure rather than by primary alterations in appetite itself.
Reduced muscle mass and strength	Catabolic factors and a lack of physical activity will cause deconditioning.	The incidence of sarcopenia increases following treatment with chemotherapy (Awad et al. 2012). Loss of lean body mass leads to a reduced capacity to exercise (Moses et al. 2004).	Sarcopenia is a significant indicator of chemotherapy toxicity and efficacy. Dose reductions and treatment delays are common in these patients (Prado et al. 2009; Prado et al. 2007). Low muscle mass has been shown to be an independent predictor of immobility and mortality (Prado et al. 2008). Reduced muscle function is associated with poorer QoL (Kilgour et al. 2013; Norman et al. 2010).
Reduced fat mass	Derangements in lipid metabolism can lead to pronounced loss of body fat.	Increased rates of fat oxidation and decreased lipogenesis have been demonstrated in cancer patients (Cao et al. 2010; Jeevanandam et al. 1986). Lipid-mobilising factor has been detected in the urine of patients with cancer and may be responsible for progressive lipid depletion (Russell & Tisdale 2002). Significant reductions in fat mass have been reported in patients with cancer following chemotherapy (Awad et al. 2012; Yip et al. 2014).	Increased mobilisation of peripheral fat and excessive oxidation of fatty acids can lead to depletion of fat stores and indirectly to weight loss (McAndrew 1986). Changes in fat composition (i.e. lower visceral to subcutaneous fat ratio) could be related to positive circumferential resection margin status following surgery (Yip et al. 2014).
Increased metabolism	Increased metabolism can lead to weight loss.	Tumour progression, comorbidities, old age, physical deconditioning, nutritional deficiency and drugs contribute to hypermetabolism in cancer (Dodson et al. 2011). Liver metastasis appears not to influence it (Cao et al. 2010).	Elevated resting energy expenditure shows a much stronger association to weight loss than energy intake does indicating that an expected up-regulation of dietary intake in

Component	Description	Research Findings	Effect
		Although inconsistent findings have been reported, cancer patients, in general, are at a higher energy consumption condition compared with healthy controls (Cao et al. 2010).	response to elevated energy expenditure is frequently lost in cancer patients.
Inflammatory factors	Imbalance between pro- and anti-inflammatory cytokines may contribute to malnutrition and cachexia.	Pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and IFN-γ have all been associated with cachexia (Espat et al. 1995; Espat et al. 1994; Strassmann & Kambayashi 1995; Staal-van den Brekel et al. 1995). Levels of IL-12 are significantly lower in cachectic patients compared with controls (Shibata et al. 2002). C-reactive protein has been associated with anorexia and hypermetabolism (Wigmore et al. 1997). There is no clear, reproducible pattern whereby the plasma concentration of any one cytokine or acute phase reactant can be directly related to cachexia (Blum et al. 2011).	These cytokines initiate a cascade of events, including direct effects on metabolism and appetite suppression and indirect effects on the acute phase protein response. This response has been associated with the increase in the resting metabolic rate and loss of muscle mass (Falconer et al. 1994; Deans & Wigmore 2005).
Catabolic factors	Cancer may induce abnormalities in substrate metabolism.	Gluconeogenesis is known to be increased in patients with cancer (van Cutsem & Arends 2005). Due to insulin resistance and glucose intolerance, this extra glucose is poorly used by peripheral tissues (Rofe et al. 1994; Mutlu & Mobarhan 2000). Patients with cancer have high rates of glycerol, free fatty acid and amino acid turnover (McAndrew 1986; Shaw & Wolfe 1987).	Such futile substrate cycling leads to an increase in energy requirements and consequently there is a noticeable depletion of body fat and protein stores (Smith & Tisdale 1993; Heber et al. 1982).
Functional effects	High levels of fatigue may negatively affect physical state.	More severe weight loss has been associated with a higher level of fatigue, which diminishes concentration and alertness (Fearon et al. 2006; Stewart et al. 2006). Cachectic patients have a marked reduction in physical activity levels (Dahele et al. 2007; Moses et al. 2004).	Increased fatigue coupled with reduced appetite can lead to a reduction in performance status in cachexia (Argilés et al. 2007).
Psychosocial effects	High levels of fatigue may negatively affect psychological state.	Eating-related anxiety, frustration, helplessness and fear are common in patients with cachexia (Reid et al. 2009). Depression has been shown to be significantly linked to cachexia (Westin et al. 1988).	Malnutrition has been found to have a detrimental effect on patient QoL due to poorer general health, reduced social functioning and a more negative outlook (Fouladiun et al. 2007; Ovesen et al. 1993; Andreyev et al. 1998).
Abbreviations: IFN-γ, interferon gamma; IL-, interleukin; QoL, quality of life; TNF-α, tumour necrosis factor alpha			

1.1.4.2 Prevalence of Malnutrition in Oncology

Researchers have been interested in assessing malnutrition in cancer patients for many decades. However, the research performed to date has generally not defined malnutrition using the international consensus approach outlined in Section 1.1.4.1. Nevertheless, using a range of different diagnostic criteria, it is reported that patients with cancer have the highest prevalence of malnutrition among hospitalised in-patients, ranging from 40-80% (Ollenschläger et al. 1991; Shike 1996; Delmore 1997).

Large studies of malnutrition prevalence have been undertaken in the UK, albeit not specifically in the oncology setting. In hospitals, care homes and mental health units, four Nutrition Screening Week Surveys were undertaken by the British Association of Parenteral and Enteral Nutrition (BAPEN) between 2007 and 2011 (Russell & Elia 2014). The surveys aimed to establish the prevalence of malnutrition using the Malnutrition Universal Screening Tool (MUST) in different care settings. This tool involves the addition of three scores: a BMI score, an unintentional weight loss score and an acute disease effect score. There were a total of 661 hospital centres and 34,699 patients involved in the surveys. Overall the prevalence of malnutrition in patients newly admitted to UK hospitals was 29% (Russell & Elia 2014). The prevalence of malnutrition was found to be higher in those with cancer (39%) compared with those without it (28%) and those patients with GI disease had a consistently higher prevalence (43%) when compared with the other diagnostic categories. A pitfall of this research, is that MUST is a screening tool rather than an in-depth nutritional assessment tool, and thus, cannot diagnose malnutrition.

Specifically in oncology, malnutrition prevalence can vary depending upon the setting. Dewys and colleagues demonstrated that the prevalence of malnutrition was high in the US out-patient setting. Patients (n= 3,047) reported unplanned weight loss in the preceding six months and this was recorded within the following ranges: 5-10% or > 10% (Dewys et al. 1980). Depending on the primary tumour site, 8-32% had lost 5-10%, while 4-38% had lost > 10% of their weight. In a more recent observational, cross-sectional study undertaken at an Australian public tertiary

hospital, the prevalence of malnutrition was measured using the valid and reliable Patient Generated Subjective Global Assessment (PG-SGA). The rates of malnutrition ranged from 26% (13/50) in the out-patient chemotherapy unit, 56% (71/126) in the cancer clinic, and 67% (10/15) in the oncology ward (Isenring et al. 2010).

By far one of the largest studies of malnutrition prevalence in cancer is the one-day prospective French prevalence survey, results of which were recently published (Hebuterne et al. 2014). Information on nutrition status was collected on 1903 cancer in- and out-patients (mixed tumour sites) in 24 cities. Malnutrition was defined as (a) BMI < 18.5 kg/m^2 in those aged < 75 years or < 21 kg/m^2 in those ≥ 75 years and/or (b) weight loss > 10% since disease onset. The nutrition risk index (NRI) screening tool was used in a sub-group of patients to identify malnutrition and risk thereof. Serum albumin is a component of the NRI equation. Overall, 39% of patients were considered malnourished using the BMI and weight loss definitions. Since disease onset, 84% of the patients had experienced weight loss, and 51% had lost more than 5% of their body weight. Of the 103 with oesophageal or gastric cancer, 60% were found to be malnourished. In the subgroup of patients (n= 368) for whom a recent serum albumin result was available, NRI was calculated and the prevalence of malnutrition was 59% (37% were moderately malnourished and 22% were severely malnourished).

Although the findings of this study highlight the high prevalence of malnutrition in the oncology setting, they must be interpreted with caution. Body mass index used as a measure of nutritional status alone is often criticised as it does not provide information on body fat distribution and body composition and is prone to error e.g. underestimation of malnutrition in obese individuals. Also, it does not take into consideration non-nutrition influences on BMI such as oedema, ascites, or a low but stable index. The use of the NRI screening tool (with serum albumin as a component) is also noteworthy (Hebuterne et al. 2014). Following a review of the literature, the Academy of Nutrition and Dietetics confirmed that serum albumin does not consistently or predictable change with weight loss, energy restriction or nitrogen balance (American Dietetic Association Evidence Library 2014). They suggest that hypoalbuminaemia

reflects the severity of the inflammatory response rather than poor nutritional status and as such is not a valid surrogate marker. However, referring back to the aetiology-based definitions discussed in Section 1.1.4.1, both the acute disease/injury-related malnutrition and the chronic-disease related malnutrition relate to inflammation. Therefore, malnutrition and inflammation can co-exist i.e. a malnourished patient is likely to have a low serum albumin. The presence of inflammation can contribute to malnutrition and can blunt a favourable response to nutritional intervention. Conversely, if inflammation is absent then even advanced malnutrition due to starvation can be reversed with appropriate nutritional support (Jensen et al. 2009; Jensen et al. 2010). Therefore, though serum albumin is not an ideal surrogate marker of nutritional status, it can provide insight into the pathophysiology of malnutrition.

Another malnutrition prevalence study involving 1,000 patients was recently published. The cohort consisted of head and neck, lung and GI cancer patients, all of whom were receiving radiotherapy. Nutritional status was measured using the validated Subjective Global Assessment (SGA) (Koom et al. 2012). In total, the mean (SD) weight loss over the previous six months was 2.1 (3.9) kg and 275 (27.5%) patients had lost some weight in the previous one month. The prevalence of malnutrition was high in the cohort at 42.6%. Of those with GI cancer (n= 444, 44.4%), 169 (43.1%) were malnourished. This value was much higher than that for the head and neck and lung cancer patients, at 29.1% and 27.8% respectively.

A number of studies have investigated nutritional risk specifically in GI cancer. In a Chinese study of advanced GI cancer patients (n= 498), the need for nutritional intervention was calculated using PG-SGA (Zhang et al. 2014). The mean (SD) PG-SGA total score was higher for oesophageal cancer patients (11.07 (4.03)) than other GI cancer groups and significantly higher than that of colon cancer (9.54 (3.50)), indicating a more critical need for improved symptom management and nutritional intervention. Nutritional status results were not reported for the cohort.

High prevalence rates for malnutrition in patients with OG cancer were found in an Italian study. The study involved almost 1,500 cancer out-patients, most of who were having ongoing oncology treatment (Bozzetti et al. 2012). One-fifth of the cohort had OG cancer. The Nutrition Risk Screening (NRS) 2002 tool was used to determine nutritional risk, where a score of 3-4 indicates that the individual is at medium risk and a score of > 5 indicates a high risk of malnutrition. The results were presented as the percentage of patients at medium risk of malnutrition: of those with oesophageal and gastric cancer, 62.5% and 43.7% respectively were at medium risk. Compared with all other cancer groups, those with oesophageal cancer had more weight loss and were at a higher nutritional risk.

In a local in-patient study at RM, the nutritional status of 128 patients was assessed using PG-SGA (Shaw et al. 2014). There were 26 (21%) GI cancer patients in the study. The PG-SGA categorised 36 (29%) as well-nourished and 90 (71%) as malnourished of which 63 (50%) were moderately/suspected malnourished and 27 (21%) were severely malnourished. Another RM prospective study added to the evidence of increased nutritional risk for patients with OG cancer. Consecutive patients (n= 920) referred for consideration of treatment of their newly diagnosed GI cancer at RM were recruited (Baldwin et al. 2006). Reported unplanned weight loss in the preceding three-six months was recorded and if the weight loss was 5-10% or > 10%, the patient was considered to be at medium or high risk of malnutrition respectively. Overall 223 (24%) patients had lost 5-10% of their body weight, while 294 (32%) had lost > 10%. In the combined gastric and oesophageal cancer group (n= 238), 24% had 5-10% weight loss, while 37% had weight loss of > 10%.

There are numerous causes for malnutrition and it is certainly prevalent in cancer with many data suggesting an increased risk of malnutrition in patients with OG cancer. However, there are few studies that have investigated its prevalence exclusively in patients with OG cancer by means of a nutritional screening or assessment tool.

1.1.4.3 Consequences of Malnutrition in Oncology

Malnutrition has been shown to have measurable and important adverse effects on clinical outcome. In patients with cancer, malnutrition has been associated with increased toxicity to oncological treatments (van Cutsem & Arends 2005; Andreyev et al. 1998), lower response to treatment (van Cutsem & Arends 2005) and lower overall survival (Dewys et al. 1980; Clavier et al. 2014). These poorer outcomes may be explained by the finding that individuals who present with weight loss receive less chemotherapy than those without weight loss because the treatment dose is calculated according to body surface area, which takes weight into account (Ross et al. 2004; Andreyev et al. 1998). Stabilisation of body weight is associated with a significantly increased survival compared with those who continue to lose weight (Andreyev et al. 1998). Malnutrition has also been shown to be strongly associated with deteriorating performance status (Hebuterne et al. 2014), it impairs immune status and reduces the body's defence against infectious diseases (Alexandre et al. 2003) and results in higher overall treatment costs by causing longer length of stay in hospital (Kyle et al. 2005).

In a systematic review assessing the role of nutritional status in predicting QoL in cancer, it was concluded that better nutritional status was positively associated with better QoL in GI cancer patients (eight studies) (Lis et al. 2012). In Carey et al.'s cross-sectional study of upper-GI cancer patients following surgery (n= 30) (refer to 1.1.3.1), nutritional status was assessed to determine if it might help to explain QoL scores (Carey et al. 2011). Mean (SD) weight loss since surgery was 9.8 (10.5) kg and those patients with malnutrition had poorer QoL; QoL was correlated with BMI ($r= 0.524$; $p= 0.004$) and percentage weight change ($r= 0.494$; $p= 0.006$) after adjustment for age. Fourteen patients (47%) displayed some degree of malnutrition using SGA. Along with GSRS this score was significant in explaining 50.3% of variance in QoL, with a high beta value of -0.458 ($p= 0.003$).

Although many studies have focused on assessing the relationship between malnutrition and other variables, this does not by any means imply causation. There are many confounding factors that need to be considered e.g. patients with weight loss have poorer survival, but this

may be because they have more advanced cancer or poorer performance status, and as such, it cannot be concluded that weight loss was the sole cause for reduced survival.

1.1.4.4 Oral Intake in Patients with Oesophagogastric Cancer

Inadequate oral intake can contribute to malnutrition in cancer. Oral intake might be compromised following surgery due to absence or reduction in the reservoir of the stomach. However, research in OG cancer patients with regard to this is limited and conflicting.

A prospective evaluation of dietary intake after near-total oesophagectomy (with or without perioperative chemotherapy) (n= 32) indicated that most patients appeared to be meeting their energy needs at a mean (SD) of 2.8 (1.8) years post-surgery (Ludwig et al. 2001). It was found that the mean (SD) energy intake was 2,179 (502) kcal/day, which was, as a group, 98% of the recommended energy intake (based on ideal body weight). Overall, 78% (25 of 32) were able to meet or exceed daily energy intake recommendations based on ideal body weight. However, it was not reported whether patients were weight stable at the point of dietary assessment, thus it is not possible to conclude that dietary adequacy prevented the development of malnutrition.

A Korean study of 20 patients suggested that dietary intake was not sufficient to maintain body weight. In this study, patients underwent a total gastrectomy and adjuvant chemotherapy and oral intake was assessed at a mean (SD) of 2.8 (2) years following the end of treatment (Bae et al. 1998). Weighed food diaries were used, although the number of assessment days was not clearly stated. The mean (SD) energy intake was 1,586 (129) kcal/day (range 883-3139 kcal/day), which was 31.8 (10.9) kcal/kg body weight (range 16.7-57.1 kcal/kg). This intake was somewhat lower than the average daily energy intake of Korean adults, which is 1,838 kcal/day. There was also a mean 15% loss of preoperative weight and fat mass (measured by triceps skinfold thickness) was significantly less than that of the normal Korean population. Although, the sample size was small, and no estimations of energy requirements were made, these results indicated that insufficient energy intake might be a cause for the weight loss and malnutrition seen in OG cancer.

In Carey et al.'s cross-sectional study of weight-losing upper-GI cancer patients six months or more after surgery (n= 30), patients completed 3-day weighed food diaries (Carey et al. 2011). The mean (SD) time since surgery was 1.3 (2.2) years. Nutritional requirements for energy were estimated using the Schofield equation, where basal requirements were multiplied by a 1.5-1.8 activity factor, depending on the reported level of activity. Protein requirements were based on standard requirements for free living individuals, with a range of 0.8-1 g/kg per day. Their data agrees with that of the Korean study: mean (SD) energy intake as a percentage of estimated daily requirements was 79 (20.6)%. However, mean (SD) protein intake appeared adequate at 118.2 (32.9)% of estimated requirements. A limitation of this study lies in the use of the Schofield equation to estimate energy requirements, which has been shown to overestimate resting energy expenditure in patients with pancreatic cancer (Bauer et al. 2004). Still, there are no prediction equations that have been specifically developed for use in cancer and although indirect calorimetry is the most accurate method for determining energy requirements, it is impractical, time-consuming and expensive in the clinical setting. In addition the small sample size limits the ability to draw firm conclusions on dietary intake.

Similarly, data from a study involving just six post-gastrectomy patients agreed with these findings for protein, although not for energy. The median (range) time of assessment in this study was somewhat longer at 3.8 (2.1-5) years (Curran & Hill 1990). Dietary intake was assessed using a 24-hour recall and a 3- or 4-day dietary diary. The patients had a mean (SD) daily protein intake of 81 (15) g/day, which was not significantly different from their estimated daily requirements of 85.7 (3.5) g/day (based on individual estimated requirements of 1.5 g/kg/day). The mean (SD) energy intake was not significantly different from their estimated requirement, 2,224 (381) kcal and 2,284 (93) kcal respectively (based on individual estimated requirements of 40 kcal/kg/day). However, pre-diagnosis weight was significantly lower than post-surgery weight ($p< 0.02$) suggesting that oral intake at diagnosis/during and after treatment (i.e. before the study assessment) was inadequate.

There are no large studies assessing dietary intake patterns in the OG cancer setting. The studies discussed above are very heterogeneous, with different methods used for assessing dietary intake and estimating energy and protein requirements. As such, one cannot be certain that inadequate oral intake is contributing to malnutrition in all weight-losing OG cancer patients.

1.1.4.5 Nutritional Psychosocial Factors in Patients with Oesophagogastric Cancer

Beyond the physical difficulties with eating and the metabolic implications of severe weight loss, the psychosocial impact of adapting to a new way of eating appears to be substantial. This has been mentioned previously in Table 1-7.

Many qualitative studies focused on patients' own experiences of changes in their appetite, food intake and weight loss. They provide us with a deep understanding of the patients' experience. In one such study, qualitative interviews were performed with 15 participants. Patients were asked to recount their experiences concerning appetite and hunger, smell and taste, changes in weight, and type of nutritional intake three months following gastrectomy (Olsson et al. 2002). Three themes were identified: the struggle to eat and drink, bodily estrangement and nutritional treatment regimens.

With reference to the first theme, the majority (proportion not known) of patients indicated that they did not have any appetite after their operation, that they did not feel hungry and that nausea was common. One patient said that he had to force himself to eat and in some cases, spouses or relatives tried to force the patients to eat more frequently, despite their lack of appetite or feelings of hunger. Regarding the second theme (bodily estrangement), the patients in this study experienced a range of variations in their weight following surgery. This affected their bodily perception and lead to feelings of alienation with their own bodies. For the third theme (nutritional treatment regimens), individuals receiving enteral nutrition (EN) felt irritated and uncomfortable because of it. They had feelings of isolation, because they could not meet their friends during the evenings and it limited their freedom (Olsson et al. 2002).

Similarly, in another qualitative study by Olsson et al. 15 patients (n= 13 had cancer) were assessed one year after upper-GI surgery. The patients related feelings of caution with regard to their diet (2010). If they ate too much at any one time or ate too quickly, it could lead to overload and vomiting. Many patients felt they could no longer eat foods high in fat. During the year they reported learning to be aware of any foods that did not agree with them and that could cause negative physical reactions. For example, some patients could not consume any meat, dairy products, or fresh vegetables. For these individuals, having the ability and desire to share a meal with others, appreciate good food, and regaining their appetite were seen as a positive recovery sign (Olsson et al. 2010).

Feelings of embarrassment about eating in public and/or being unable to control how their body would react when eating (e.g. nausea, vomiting) has been described in another focus-group study (Malmström et al. 2013). Bodily perception was also subject to change in this group. Participants reported feeling as though they had become shorter, more compact, or shrunken and thus, had become alienated from their body (Malmström et al. 2013).

The effects of nutrition-related problems are far-reaching: malnutrition is the obvious factor to consider but the psychosocial effects should not be overlooked in an effort to prevent QoL decline.

1.1.5 Research Needs in Patients with Oesophagogastric Cancer: Gastrointestinal Symptoms and Nutritional Status

Acute and chronic GI symptoms have not been studied systematically or prospectively in OG cancer patients undergoing radical treatment and, as such, the symptom burden is not fully understood. Also, these patients represent a high-risk group for malnutrition, yet there is still only a relatively small body of research concerned with measuring their nutritional status. Furthermore, although GI symptoms and nutritional status are theoretically likely to be associated with each other, this has only been investigated by a few studies, as described in Table 1-8. Of these studies, none have incorporated the use of an oncology-specific nutritional

assessment tool and the number of GI symptoms measured is generally small. Of note, just one pilot study looked at this relationship in upper-GI cancer patients and there is no data for OG cancer specifically (Chate 2006).

There has also been limited research to assess the prevalence of SIBO in an OG cancer population. The few studies that have focused on this have involved patients following gastrectomy and high prevalence rates (37-100%) were reported (Paik et al. 2011; Iivonen et al. 1998; Brägelmann et al. 1997). To date, no researchers have tested for SIBO before the commencement of treatment and then re-tested during and after treatment to determine the incidence of the condition at these points. Therefore, a gap in the literature remains, which, if filled, could potentially change the management of GI symptoms in OG cancer patients.

Table 1-8 Studies investigating the relationship between gastrointestinal symptoms and nutritional status in patients with cancer

Reference	Study design, cohort	n=	Gastrointestinal symptom and nutritional status assessment	Findings
Sánchez-Lara et al. 2012	Retrospective study, mixture of cancers, out-patients, attending chemotherapy clinics	191	5 symptoms: anorexia, nausea, vomiting, diarrhoea, constipation 6 month unintentional weight loss	Most commonly reported symptoms were nausea (60%) and anorexia (46%) 39% had ≥ 5% weight loss, 25% had ≥ 10 % weight loss In patients with ≥ 5% weight loss, there was a significant associated with nausea (p= 0.03), vomiting (p= 0.017) and anorexia (p= 0.003). In those with ≥ 10% weight loss, there was a significant associated with vomiting (p= 0.05) and anorexia (p< 0.001)
Bovio et al. 2009	Cross-sectional study, mixture of cancers, advanced disease, no active treatment	143	10 symptoms: anorexia, dry mouth, dysphagia to solids, dysphagia to liquids, decreased taste, taste changes, chewing problems, nausea, vomiting, hiccup 6 month unintentional weight loss, triceps skinfold thickness, mid-arm muscle circumference and BMI	Most commonly reported symptoms were dry mouth (73%), anorexia (50%), chewing problems (40%), hiccup (20%) and nausea and vomiting (31%) 54% had > 10% weight loss Weight loss was significantly associated with anorexia (p= 0.03), dysphagia to solids (p= 0.04) and liquids (p< 0.001) and nausea (p= 0.025). Those with > 10% weight loss had a relative risk for developing dysphagia to liquids of 3.94 compared with those with < 10% weight loss
Khalid et al. 2007	Cross-sectional study, GI and lung cancer, any disease stage, out-patients, recent surgery in 18%	151	12 symptoms: anorexia, dry mouth, mouth sores, dysphagia, taste changes, bothersome smells, early satiety, nausea, vomiting, pain, diarrhoea, constipation PG-SGA (only first part of tool used),	62% had symptoms: most commonly reported symptoms were anorexia (38%), early satiety (27%), pain (23%), taste changes (20%) and nausea (18%) 48% and 28% of those with GI and lung cancer had lost weight

Reference	Study design, cohort	n=	Gastrointestinal symptom and nutritional status assessment	Findings
			including weight loss over past 1-6 months	Those with 5% or more weight loss had more symptoms than patients with no weight loss (p< 0.0001). Those with any weight loss were significantly more likely to report anorexia and dysphagia than those without weight loss (p= 0.002 and p= 0.032)
Chate 2006	Cross-sectional study, upper-GI cancer, disease stage not reported, out-patients, treatment modalities not reported	40	10 symptoms: anorexia, dry/sore mouth, dysphagia, taste changes, early satiety, heartburn, nausea, abdominal pain, diarrhoea, constipation 3 month unintentional weight loss, BMI	Most commonly reported symptoms were anorexia (45%), early satiety (35%), abdominal pain (33%), dysphagia (30%) and nausea (30%) 65% had lost some weight The weight loss group reported a greater number of symptoms especially anorexia (54%) and early satiety (42%). Nausea (46%) and dysphagia (42%) in the weight loss group was significantly different compared with the non-weight loss group (p≥ 0.05)
Petruson et al. 2005	3-year longitudinal study, head and neck cancer, any disease stage, out-patients, multimodality treatment in 81%	49	8 symptoms: anorexia, dry mouth, sticky saliva, dysphagia, nausea, vomiting, diarrhoea, constipation 6 month unintentional weight loss	41% had lost ≥ 10% body weight and 59% had lost < 10% In those with ≥ 10% weight loss compared with those with less weight loss, symptoms were significantly worse at: (a) Diagnosis: anorexia (p< 0.01), dysphagia (p< 0.01), dry mouth (p< 0.05), sticky saliva (p< 0.001) (b) 1 year: sticky saliva (p< 0.05) (c) 3 years: anorexia (p< 0.05), dry mouth (p< 0.05)

Reference	Study design, cohort	n=	Gastrointestinal symptom and nutritional status assessment	Findings
Sarhill 2003	Cross-sectional study, mixture of cancers, metastatic disease, in- and out-patients, recent chemotherapy in 24%, radiotherapy in 28%	352	15 symptoms: anorexia, dry mouth, sore mouth/throat, odynophagia, decreased taste, early satiety, belching, hiccup, nausea, bloating, vomiting, abdominal pain, dyspepsia, diarrhoea, constipation 6 month weight loss, triceps skinfold thickness, mid-arm muscle circumference and BMI	Most commonly reported symptoms were anorexia (81%), early satiety (69%), dry mouth (69%), constipation (59%) and nausea (49%) 87% had lost some weight, and most (71%) lost ≥ 10% of pre-illness weight The absolute number of GI symptoms correlated (r= 0.8) with severity of weight loss (p= 0.01)
Grosvenor et al. 1989	Cross-sectional, mixture of cancers, unresectable disease, recent chemotherapy in 36%, recent radiotherapy in 54%	254	12 symptoms: dry mouth, sore mouth, difficulty chewing, taste changes, dysphagia, nausea, abdominal fullness, abdominal pain, milk product intolerance, vomiting, diarrhoea, constipation Weight loss compared with usual weight (no time frame), triceps skinfold thickness, mid-arm muscle circumference	Most commonly reported symptoms were abdominal fullness (61%), taste change (46%), constipation (41%), dry mouth (41%) and nausea (39%) Mean weight loss of 12% from their usual body weight, 67% had lost > 5% Symptoms significantly more common in the group with > 5% weight loss included abdominal fullness (p< 0.001), taste change (p< 0.003), vomiting (p< 0.005), and dry mouth (p< 0.02)
Abbreviations: BMI, body mass index; GI, gastrointestinal; PG-SGA, Patient-Generated Subjective Global Assessment				

1.2 Nutritional Screening, Assessment and Intervention in Patients with Oesophagogastric Cancer

Malnutrition is a common phenomenon in patients with OG cancer. It can occur at diagnosis, during treatment and/or after the completion of treatment. The optimisation of nutritional status can be achieved when three essential components of a nutrition support programme are effectively implemented: screening, assessment and intervention. The aim of screening is to quickly identify individuals at nutritional risk so that they may undergo a more formal and extensive nutrition assessment. If a patient is found to be malnourished on assessment, an appropriate nutritional intervention should be started with the aim of improving nutritional status, clinical outcomes and QoL.

1.2.1 Nutritional Screening in Patients with Oesophagogastric Cancer

The 2003 ESPEN guidelines state that the purpose of nutrition screening is to predict the probability of a better or worse outcome due to nutrition factors and whether nutrition treatment is likely to influence this (Kondrup, Allison, et al. 2003b). Routine nutrition screening has been endorsed by many national, international and specialist organisations including the National Institute for Health and Care Excellence (National Institute for Health and Care Excellence 2006), the British Dietetic Association (BDA) (The British Dietetic Association 1997), BAPEN (Elia 2003), ESPEN (Kondrup, Allison, et al. 2003b), the Council of Europe (Beck 2001) and the Royal College of Physicians (The Royal College of Physicians London 2002) .

Screening is often performed by a non-expert in nutrition, such as a nurse or healthcare assistant (Green & Watson 2005). More recently, researchers have investigated patient self-screening, including electronic self-screening, to good effect (Cawood et al. 2012; McGurk et al. 2013). Screening should be a non-invasive, quick and simple process. To efficiently screen a patient's nutrition status, readily available objective and subjective data are reviewed. Height, weight, weight change, primary diagnosis, illness severity, change in food intake and the presence of symptoms are objective measures commonly included in nutritional screening tools (Huhmann & August 2008). For patients with cancer, it has been proposed that nutritional

screening should be performed at diagnosis, initiation of treatment and at scheduled intervals throughout treatment to monitor changes in nutritional status (Huhmann & Cunningham 2005). There are a variety of nutritional screening tools, of which the NRI, NRS 2002, Malnutrition Screening Tool (MST), MUST, Malnutrition Screening Tool for Cancer (MSTC) and Mini Nutritional Assessment (MNA) are frequently used and investigated (Green & Watson 2005). A brief description of these tools is described in Table 1-9.

Table 1-9 Overview of commonly used nutritional screening tools

Tool	Components	Validation cohort(s)
Nutrition Risk Index	Equation: NRI= 1.519 (serum albumin; g/dL) + 41.7(current weight/usual weight)	Surgical patients
Nutrition Risk Screening 2002	4 items: BMI, weight loss, dietary intake, illness severity	General hospital in-patients, acute hospitalised patients
Malnutrition Screening Tool	3 items: weight, percentage weight loss, appetite	Acute hospitalised patients, elderly care home residents, cancer patients receiving radiotherapy, cancer patients receiving chemotherapy
Malnutrition Universal Screening Tool	3 items: BMI, percentage weight loss, acute disease effect	General hospital in-patients, out-patients and community dwellers
Malnutrition Screening Tool for Cancer	Equation: MSTC= -0.116 + (1.777 × intake change) + (1.304 × ECOG performance status) + (1.568 × weight loss) + (-0.187 × BMI)	Cancer in-patients
Mini Nutritional Assessment	6 items: dietary intake, weight loss, mobility, psychological stress/acute disease, neuropsychological problems, BMI	Elderly in-patients, out-patients and elderly community dwellers
Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; MSTC, Malnutrition Screening Tool for Cancer; NRI, Nutrition Risk Index References: Kondrup, Rasmussen, et al. 2003a; Ferguson, Capra, et al. 1999a; Stratton et al. 2004; Kim et al. 2011; Guigoz & Vellas 1999; The Veterans Affairs Total Parenteral Nutrition Cooperative Study Group 1991; Bauer et al. 2005; Ferguson, Bauer, et al. 1999b; Isenring et al. 2006; Isenring et al. 2009		

The majority of these screening tools were developed for general hospitalised or community patients, with MSTC being the only one designed specifically for cancer in-patients. As such, few tools have been validated in cancer patients. To date, seven well-conducted studies have aimed to validate a nutritional screening tool in adult oncology against an acceptable reference standard (i.e. SGA or PG-SGA) with five studies indicating suitability of four screening tools tested (Table 1-10).

Table 1-10 Studies measuring the validity of nutritional screening tools in adult patients with cancer against Subjective Global Assessment (SGA) or Patient Generated Subjective Global Assessment (PG-SGA)

Reference	Study cohort	Tool tested	Reference standard	Sample size, n=	Malnutrition (%)	Sensitivity (%) of tool	Specificity (%) of tool	Suitability of tool *
Read et al. 2005	Out-patients, mixture of cancers, prior treatment in the majority	MNA	PG-SGA	157	65	97 (59% PPV)	54 (NPV n/a)	Not suitable
Ferguson, Bauer, et al. 1999b	Out-patients, mixture of cancers, receiving radiotherapy	MST	SGA	106	11	100 (40% PPV)	81 (100% NPV)	Suitable
Isenring et al. 2006	Out-patients, mixture of cancers, receiving chemotherapy	MST	PG-SGA	50	26	100 (80% PPV)	92 (100% NPV)	Suitable
Wolf et al. 2002	In-patients, gynecological cancers, treatment not described	MST	SGA	96	21	80 (46% PPV)	75 (100% NPV)	Not suitable
Kim et al. 2011	In-patients, mixture of cancers, treatment not described	MSTC	PG-SGA	257	67	94 (68% PPV)	84 (98% NPV)	Suitable
Boléo-Tomé et al. 2011	Out-patients, mixture of cancers, receiving radiotherapy	MUST	PG-SGA	450	29	80 (87% PPV)	89 (100% NPV)	Suitable
Bauer & Capra 2003	In-patients, cancer site and treatment not described	MAG	SGA	65	75	59 (88% PPV)	75 (38% NPV)	Not suitable
Shaw et al. 2014	In-patients, mixture of cancers, treatment not described	RMNST MST	PG-SGA PG-SGA	128	71	93 (83% PPV) 66 (91% PPV)	53 (53% NPV) 83 (49% NPV)	Suitable Not suitable

* Suitability of the tool: as determined by the study investigators. Abbreviations: MAG, Malnutrition Advisory Group tool; MNA, Mini Nutritional Assessment; MST, Malnutrition Screening Tool; MSTC, Malnutrition Screening Tool for Cancer; MUST, Malnutrition Universal Screening Tool; n/a, not available; NPV, negative predictive value; PPV, positive predictive value; RMNST, Royal Marsden Nutrition Screening Tool

1.2.2 Nutritional Assessment in Patients with Oesophagogastric Cancer

Nutritional screening allows identification of patients at risk of malnutrition. For those at risk, a comprehensive nutritional assessment should be performed by an expert in clinical nutrition (e.g. a dietitian or nutrition-trained doctor). Nutritional assessment incorporates medical and weight history, a detailed dietary history, physical examination, anthropometric measurements and laboratory data (American Dietetic Association Council on Practice Quality Management Committee 1994). An assessment of body compartments as well as an analysis of structure and function of organ systems and metabolic status is included (Huhmann & August 2008). The purpose of nutritional assessment is to collect the information necessary to establish nutrition-related diagnoses and to formulate nutrition, metabolic, dietary, pharmacologic, and functional interventions in the form of a nutrition care plan (Huhmann & August 2008).

Many nutritional assessment tools have been developed that combine the aspects of nutritional assessment into an algorithm or score, although few have been validated in cancer patients. Two tools that have been evaluated in prospective trials of cancer patients and have been shown to have adequate sensitivity and specificity: SGA and PG-SGA (Detsky et al. 1987; Bauer et al. 2002; Ottery 2000).

The SGA was originally developed in the 1980's for GI surgical patients and is comprised of history (weight loss, dietary intake, GI symptoms and functional capacity), metabolic demands of the underlying disease and a nutrition-related physical examination (Detsky et al. 1987). The rating of nutritional status as (a) SGA-A, well-nourished, (b) SGA-B, moderately/suspected malnourished or (c) SGA-C, severely malnourished is subjective. The SGA tool was subsequently modified by Ottery and Bauer and PG-SGA has evolved. Ottery modified the SGA making it specific for an oncology population (Ottery 1994). The history section of the tool became *'patient-generated'* to simplify the process and to involve the patient more. Also, the number of GI symptoms in this section was increased to include nutrition-impact symptoms relevant to cancer patients (Ottery 1994). Then, Bauer added a scoring and triage component producing the tool in use today (Bauer et al. 2002; Ottery 2000) (Appendix 8.3). The PG-SGA

tool is often referred to as the gold-standard method for nutritional assessment and is the most commonly used technique to diagnose malnutrition in practice and research. In Table 1-6, the six diagnostic criteria for malnutrition in cancer were listed and all of these items are measured by the PG-SGA.

A study of 71 cancer in-patients compared the performance of the PG-SGA with SGA and found that there was a significant difference in the median PG-SGA total scores for each of the SGA classifications ($p< 0.001$), with the severely malnourished patients (SGA-C) having the highest scores, as would be expected (Bauer et al. 2002). These findings are consistent with other studies that have shown a linear association between PG-SGA total score and SGA global category (Desbrow et al. 2005; Isenring et al. 2003). To move one SGA category (i.e. improvement or deterioration), a mean change in PG-SGA total score of ± 9.0 (95% CI= 7.2-10.9) was required (Isenring et al. 2003). In addition, the PG-SGA total score has been shown to be significantly correlated with percentage weight loss in the previous six months ($r= 0.31$, $p= 0.012$) (Bauer et al. 2002). This correlation between SGA category and PG-SGA total scores reassures that PG-SGA total score can be used as a valid measure of nutritional status.

1.2.3 Nutritional Intervention in Patients with Oesophagogastric Cancer

Formal nutrition assessment in at-risk patients identifies opportunities for dietary interventions. By providing early nutrition intervention, it may be possible to prevent or delay deterioration in patients' nutritional status (Marín Caro et al. 2007; Ravasco, Monteiro-Grillo, Vidal & Camilo 2005a; Ravasco, Monteiro-Grillo, Vidal & Camilo 2005b). A systematic review of the literature performed reported that dietary advice for adults with disease-related malnutrition (including cancer) had positive effects on weight, body composition, and hand grip strength (Baldwin & Weekes 2011). However, the effect of dietary intervention on patients with OG cancer has been researched much less than other cancer sites. Therefore, data available for this group is limited.

A two-year prospective Swedish study assessed 133 patients with colorectal and gastric cancer. Newly diagnosed patients were randomised in a 2 × 2 design between (a) nutritional support

only, (b) group rehabilitation only, (c) nutritional support and group rehabilitation or, (d) standard care (Persson et al. 2002). The individually designed nutritional support consisted of intensified primary care from nurses, psychological support from psychologists and nutritional support from dietitians. Patients who received nutritional support managed to gain weight more rapidly and to a greater extent than patients who received no nutritional support (exact data not given) (Persson et al. 2002).

The effects of a patient participation based dietary intervention on patients' nutritional and functional status following gastrectomy were determined in a 12-week randomised controlled trial (n= 48) (Kim et al. 2014). Patients were randomised to either the experimental arm (coaching through education by research nurses) or the control arm (standard care). In the experimental group, the patients received individualised dietary plans, which were approved by dietitians. At the end of the intervention, using the results from a 3-day food diary and a dietary history, participants in the experimental group consumed significantly more energy than those in the standard care group ($p= 0.001$). Body weight and BMI were falling trends in the control group, unlike in the intervention group. However, the differences were not statistically significant over time. Similarly, for nutritional status, scores from the PG-SGA only differed significantly across group over time, without any of the interaction effect (Kim et al. 2014). Of note, there was a significant difference between the groups with regard to socioeconomic background, with those in the control group tending to be socioeconomically working class ($p= 0.015$). In the intervention group, most participants were socioeconomically middle class and above, and as such, these patients may have had a good pre-intervention knowledge of nutrition and high motivation to adhere to their individual dietary plan. This is a confounding factor to consider when interpreting the positive dietary outcomes of the intervention.

Research conducted to date generally demonstrates the benefit of nutritional support in cancer with regard to macronutrient intake and weight gain. Also, as well as evidence that early nutritional intervention (when tumour burden is still limited) is able to achieve a clinical benefit with regard to nutritional status, there are also indications that it leads to improved functional

status, symptom-induced morbidity and QoL (Isenring et al. 2004; Ravasco, Monteiro-Grillo, Vidal & Camilo 2005b; Ravasco, Monteiro-Grillo, Vidal & Camilo 2005a). Given this evidence, it is of paramount importance that appropriate nutritional screening tools are used, so that those who are actually malnourished can be identified and dietetic support provided in a timely manner.

1.2.4 Research Needs for Nutritional Screening in Patients with Oesophagogastric Cancer

There is a need to ensure that nutritional screening tools used in patients with OG cancer are sensitive and specific in this setting. The MUST tool is the most commonly used screening tool in the UK but has only been validated in one cancer study (Table 1-10). In this study, Bolé-Tomé's cohort included non-selected cancer patients receiving radiotherapy and so the results should not be generalised to OG cancer patients who are treatment naïve. As such, there is no tool that can accurately indicate which patients with OG cancer need to undergo nutritional assessment. This means that there can be either (a) an overestimation of those at risk, resulting in an unnecessary strain on dietetic resources following excessive referrals or (b) an underestimation of those at risk, resulting in a lack of nutritional intervention in vulnerable patients.

1.3 Exploring the Potential of Metabolomics Technology in Small Intestinal Bacterial Overgrowth Diagnosis

1.3.1 Current Diagnostic Tests for Small Intestinal Bacterial Overgrowth

As mentioned in Section 1.1.3.2.5, there is no gold-standard test for the diagnosis of SIBO. There are three common approaches towards diagnosing the condition: the first is the traditional approach of classifying it in quantitative terms in a microbiological context; the second is the breath testing technique using carbohydrates; the third uses the symptomatic response to a trial of antibiotics.

Bacteriological analysis of small bowel secretions is a direct method of assessing the microbial populations therein. The patient undergoes an OGD during which fluid (usually 2-5 ml) is aspirated from the lumen of either the duodenum or the jejunum (usually at only one site) and then undergoes quantification in a microbiology laboratory. Traditionally, many authors have regarded this direct aspiration and culture technique as the gold-standard approach for diagnosing SIBO (Kerlin & Wong 1988; Corazza et al. 1990). However, in their systematic review, Khoshini et al. determined that it was not a gold-standard after applying the criteria of Reid et al. for the development and application of a diagnostic test (Khoshini et al. 2008; Reid et al. 1995). Of Reid's seven methodological standards, the standards that were not met by any of the studies were (a) reporting frequency and management of indeterminate results when calculating test indexes and (b) specifying test reproducibility. In addition, three of the standards infrequently met were (a) specifying spectrum of evaluated patients, (b) avoiding review bias and (c) having an adequate sample size to calculate sensitivity and specificity or likelihood ratios (Khoshini et al. 2008).

In recent years, owing to the invasive nature of the direct aspiration and culture technique, indirect tests have been developed and are now commonly used alternatives. Breath testing is the most common indirect method for evaluating SIBO (Khoshini et al. 2008). Breath tests have advantages over the direct culture method, in that they are simple to use, cheap and non-invasive. Hydrogen-based breath tests are currently the most popular and work on the assumption that the only source of H_2 production in the body is from fermentation of carbohydrates by GI microbiota.

The most frequently used substrates in breath tests are glucose and lactulose, with the former suggested to have a greater diagnostic accuracy than the latter. When compared with the direct aspiration method (i.e. taking it to be a gold-standard, although it is not), the glucose-H_2 breath test has a sensitivity of 62.5% and a specificity of 81.7% (Gasbarrini et al. 2009). The lactulose-H_2 breath test has a sensitivity of 52.4% and a specificity of 85.7% when compared with the direct aspiration method (Gasbarrini et al. 2009). Due to the high variability in oro-caecal transit

time in health and disease, combining the lactulose-H_2 breath test with scintigraphic measurement of oro-caecal transit is likely to provide a more accurate and reproducible test for SIBO (Zhao et al. 2014). However, with culture being a poor gold-standard, the validation of any breath test against it cannot be acceptably performed. Nevertheless, Grade IIA evidence suggests that the glucose-H_2 breath test is the most accurate H_2-breath test for non-invasive diagnosis of SIBO (Gasbarrini et al. 2009).

The third approach towards SIBO diagnosis is to treat it when symptoms and/or non-invasive surrogate markers (e.g. serum folate, vitamin B_{12}, haemoglobin and weight) are clinically suggestive of SIBO and to use the clinical response to antibiotics as an affirmation of SIBO being the cause of the patient's complaints- the so-called 'therapeutic trial' (Pimentel et al. 2000). With the problems associated with culture and breath testing methods (Table 1-11) it is unsurprising that Khoshini et al. found that almost one third of studies used this therapeutic trial approach for diagnostic purposes. There is, however, no standardised approach towards the type, dose or duration of the antibiotics and reported clinical response rates range from 35% to 100% (Khoshini et al. 2008).

All of the commonly used methods of diagnosing SIBO have inherent limitations as outlined in Table 1-11. As such, clinicians should be cautious when interpreting the results of such tests. Often two or three of these techniques are combined for a more robust approach, which aids clinical decision-making. For example, a therapeutic trial can be used in association with other diagnostic tests, i.e. all of the following could be taken into consideration, so as to confirm the presence of SIBO: abnormal GI symptoms/non-invasive surrogate markers, abnormal test(s) (breath test and/or aspirate and culture) and clinical response to antibiotics. Measuring response necessitates assessing symptom change systematically, as well as the resolution of abnormal parameters such as low serum vitamin B_{12} concentrations or low body weight.

Table 1-11 Limitations associated with the three common diagnostic techniques for small intestinal bacterial overgrowth

Small bowel aspiration and culture technique
• Invasive and cumbersome • Time-consuming and expensive • Technical difficulties with transport and culture of the aspirate • No consensus on sample handling and microbiological techniques • Appropriate use of anaerobic techniques is necessary • Representation of the sample is unknown • Oral bacteria may contaminate the aspirate • No consensus for defining SIBO in quantitative terms • SIBO occurring more distally in the small intestine may be missed • False negative results may occur where SIBO is caused by obligate anaerobes • Location of sampling and the amount of fluid recovered can be variable
Breath testing technique
• Low fibre diet required for 24 hours before the test • Smoking, sleep and exercise can affect test accuracy • Antibiotics and laxatives need to be avoided before the test • No consensus on a definition for a positive test (regardless of the substrate used) • Breath sampling frequency is highly variable • Both H_2 and CH_4 gases should be measured • Luminal pH differences affects carbohydrate metabolism • Carbohydrate malabsorption may lead to false positive result • Consideration of oropharyngeal bacteria • Rapid transit can give a false positive, slow transit can give a false negative • Inconsistencies in the definition of an *'early peak'* in lactulose breath testing
Therapeutic trial approach
• Follow-up may be difficult • No standardised approach towards the type, dose or duration of the antibiotic regimen • No consensus on the meaning of a clinical response to antibiotics • Over-prescribing of antibiotics • Risk of serious side-effects from antibiotic treatment • Difficulty in identifying patients without SIBO versus those with SIBO caused by an antibiotic resistant organism
Abbreviations: CH_4, methane; SIBO, small intestinal bacterial overgrowth

The dysbiosis of GI microbiota in SIBO are evidently difficult to characterise in clinical practice. Although advances in genomic technology allow for phylogenetic analysis and typing in the research setting, such methods are labourious, expensive and not suitable for routine clinical application. More accessible means of gaining insight into the dysbiosis associated with SIBO include metabolic profiling of biofluids using Metabolomics technology.

1.3.1.1 Metabolomics Technology

Metabolomics is the comprehensive assessment of all the small-molecules (metabolites) within a biological sample and attempts to systematically identify and quantify them. A metabolite is a

substance made or used when an organism breaks down food, drugs or chemicals, or its own tissue (e.g. fat or muscle tissue). Metabolomics technology enables the simultaneous identification and monitoring of a wide range of low molecular weight compounds and thus provides a biochemical fingerprint of an organism. It offers the potential for a holistic approach to clinical medicine and improving disease diagnosis, as well as, understanding disease mechanisms (Nicholson et al. 1999; Nicholson & Lindon 2008). Metabolomic studies generally use biological fluid (biofluid), cells or tissue extracts as the source of biochemical fingerprint data. The biofluids are usually relatively easy to obtain, which is an advantage in human studies. In these fluids, metabolites are in dynamic equilibrium with those inside cells and tissues and if there is an abnormal cellular process occurring in tissues of the whole organism, this will be reflected in altered biofluid compositions (Nicholson et al. 1999).

Urine and plasma are the most commonly used biofluids, but others have been studied in this context including amniotic fluid, dialysis fluids, lung aspirates, seminal fluid, synovial fluid, saliva and other digestive fluids. Of note, the largest and most complex microbiome (microbiota associated with a host) resides in the gut and interacts with the human genotype and environment to maintain body homeostasis (Qin et al. 2010). This homeostasis is hugely important, as the microbiota directly communicate with the human host e.g. controlling intestinal epithelial proliferation through toll-like receptors, enteric nerve system signalling and initiating fat storage (Tilg 2010; Abreu 2010; Sharkey & Savidge 2014). Therefore, measuring the metabolites from the host and gut microbiome together may provide an insight into any perturbations in this homeostasis.

A variety of analytical technologies have been applied to metabolomics but the most popular are nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy. These techniques have different strengths and weaknesses and can give complementary information. Nuclear magnetic resonance spectroscopy is suitable for metabolomics as it requires little physical or chemical treatment preparation, is rapid and requires only small amounts of sample/specimen. Mass spectroscopy studies, on the other hand, usually require larger amounts of the sample,

with pre-separation of the metabolites from the biological fluid needed before analysis, typically by using high-performance liquid chromatography. Liquid chromatography greatly improves the resolution characteristics and capabilities of this approach (Nicholson et al. 2005). Alternatively, the metabolites can be chemically modified to make them more volatile and so gas chromatography-mass spectroscopy can be used (Nicholson & Lindon 2008). The inherent sensitivity of this approach is useful in the detection of low concentrations of metabolites.

1.3.1.1.1 Nuclear Magnetic Resonance Spectroscopy-Based Metabolomics

Nuclear magnetic resonance-based metabolomics is a quantitative nondestructive technique that provides detailed information based on atom-centred nuclear interactions and properties (Beckonert et al. 2007). It is generally used to detect H_1 or carbon atoms in metabolites, but other atoms can also be used e.g. phosphorus, sodium, boron or lithium. Taking H_1 as an example i.e. hydrogen nuclear magnetic resonance (^{1}H NMR) spectroscopy: in a typical biofluid sample, all H_1-containing molecules in the sample without prejudice (including nearly all metabolites) will give a ^{1}H NMR signal, assuming they are present in concentrations above the detection limit. As such, the ^{1}H NMR spectrum of a biological fluid is the superposition of the spectra of all of the metabolites in the sample (Nicholson & Lindon 2008). Hydrogen nuclear magnetic resonance spectroscopy-based metabolomics using biofluids has shown high reproducibility and so, generally it is sufficient to have one sample per study time point (Keun et al. 2002; Dumas et al. 2006).

A typical ^{1}H NMR spectrum is extremely complex, consisting of thousands of peaks. The peak integrals relate directly to the number of protons giving rise to the peak, and hence to the relative concentrations of the predominantly low molecular weight metabolites in the sample (Beckonert et al. 2007). Given the complexity of the raw data, all metabolomic studies result in complicated multivariate data sets, which require visualisation software and bioinformatics methods for interpretation. Then, the complex set of biomarkers that define the biological or clinical context and help to explain the disease or tissue damage can be identified (Beckonert et al. 2007).

Multivariate statistical techniques coupled with NMR have been incorporated into the metabolomics approach to enable exploration by separation, detection, characterisation, and quantification of small molecules and related metabolic pathways of the onset and progression of human diseases. One of the most frequently used techniques in multivariate analysis is a technique called Pattern Recognition. This is a term applied to methods of data analysis that can reduce the many parameters (Nicholson et al. 1999). One of the most useful and easily applied pattern recognition techniques is principal components analysis (PCA).

Principal component analysis is an unsupervised data analysis (dimension reducing) method that can deal with large volumes and is often performed as a part of an exploratory data analysis. Principal components (PC) are new variables created from linear combinations of the starting variables with suitably weighted coefficients. The properties of these PCs are such that (a) each PC is uncorrelated with all other PCs and (b) the first PC contains the largest part of the variance of the dataset with subsequent PCs containing consistently smaller amounts of variance. Principal component analysis refers to the process by which PCs are computed, and the subsequent use of these PCs in understanding the data (James et al. 2013). It converts the multidimensional data space into a low-dimensional model plane resulting in two matrices, where the first two or three PCs give the *'best'* representation in terms of biochemical variation in the data set. Such PC maps can also serve as a tool for data visualisation i.e. visualisation of the observations or visualisation of the variables to assess for clustering. Hierarchical cluster analysis is another unsupervised method that is widely used in metabolomics data analysis, and this method has the ability to group samples according to their similarity.

The workflow for many clinical metabolomics studies using NMR spectroscopy is as follows (Zhang et al. 2013) (Figure 1-2):

- Samples are collected in a uniform way to minimise variability
- Samples are analysed by NMR to collect data on all metabolites potentially present in the sample

- Pattern recognition approaches include PCA, partial least squares discriminant analysis (PLSDA), heat map, orthogonal projections to latent structures, support vector machines, random forests method, and other methods aiming to highlight underlying trends and visualisation tools are utilised
- Trend and box plots are used to further evaluate the techniques above
- Receiver operating characteristic (ROC) curves are usually considered the method of choice for evaluating the performance of potential biomarkers

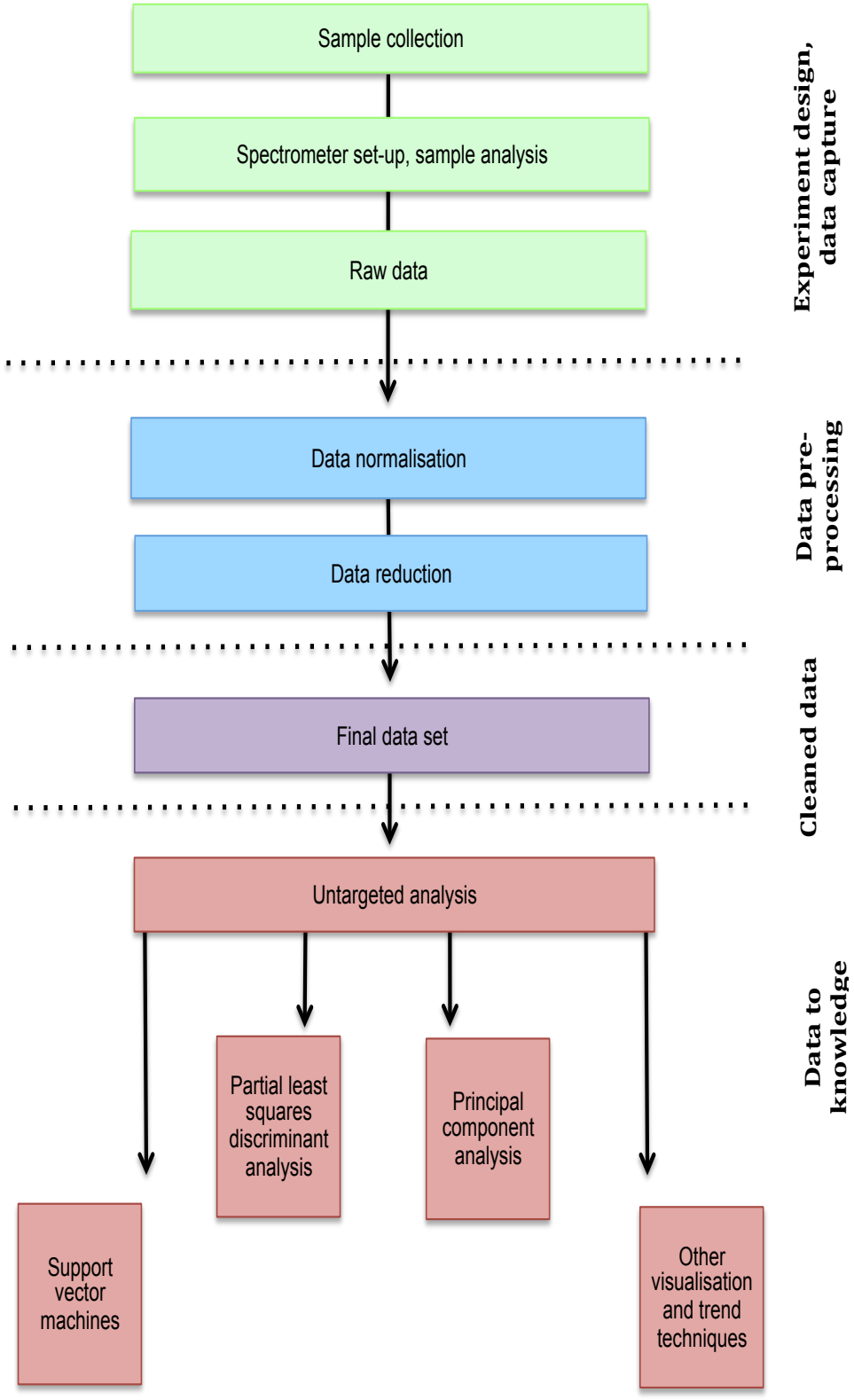


Figure 1-2 Workflow for clinical metabolomics studies using nuclear magnetic resonance spectroscopy

1.3.1.1.2 Application of Nuclear Magnetic Resonance Spectroscopy Technology

Nuclear magnetic resonance based metabolomics is becoming a useful tool in the study of biofluids and has a strong potential to contribute to disease diagnosis. The research conducted so far provides compelling evidence that this analytical platform offers great promise for the minimally invasive screening of disease-related perturbations. Metabolomics has found application not only in the study of many diseases (Brindle et al. 2002; Moolenaar et al. 2003) but also of factors such as nutrition and gut microbiota (Nicholls et al. 2003; Nicholson et al. 2005).

In a Danish study, ^{1}H NMR was used to profile the serum of patients with metastatic colorectal cancer and to determine whether a disease signature may exist that is strong enough to predict overall survival (Bertini et al. 2012). One hundred and fifty three cancer patients and 139 healthy controls were recruited. A clear metabolic signature of the disease was found to exist in the serum of the cancer patients; in the validation set, 96.7% of subjects were correctly classified. A number of metabolites were identified from the spectra, whose concentrations differed in the cancer patients and the healthy subjects; cancer patients had lower serum levels ($p< 0.05$) of alanine, citrate, creatine, glutamine, lactate, leucine, pyruvate, tyrosine and valine and higher serum levels ($p< 0.05$) of 3-hydroxybutyrate, acetate, formate, glycerol, lipid (-CH2-OCOR), N-acetyl signal of glycoproteins, phenylalanine, and proline. In addition, the results demonstrated the capability of ^{1}H NMR profiling to predict overall survival in these patients. There was a clear signature in serum in those patients with a good performance (i.e. Eastern Cooperative Oncology Group (ECOG) performance status= 0).

Another metabolomics study investigating the content of upper-small bowel (location not defined) aspirates in patients with malabsorption syndrome found that individuals with the syndrome had significantly higher median quantities of bile acids/cholesterol, acetate, formate and lactate than controls (Bala et al. 2006). In those who had both the syndrome and SIBO, significantly greater quantities of acetate, formate, lactate and unconjugated bile acids were

found compared with the controls ($p < 0.01$ for all), implying that SIBO itself might elicit a specific, potentially diagnostic metabolomic signature.

A different study using faecal samples from patients with inflammatory bowel disease (n= 10 Crohn's disease and n= 10 ulcerative colitis) and healthy controls (n= 13), also employed the metabolomics approach to aid with diagnosis (Marchesi et al. 2007). Marchesi and co-workers reported that the faecal samples obtained from the patients with Crohn's disease and ulcerative colitis manifested similar global differences in metabolic profiles compared with the healthy subjects. A depletion of short-chain fatty acids, including acetate and butyrate, was a prominent feature of Crohn's disease patients when compared with healthy subjects. In addition, a high concentration of glycerol was found in the faeces of Crohn's disease patients in comparison to ulcerative colitis patients. Higher concentrations of amino acids were also found in the faeces of patients with both Crohn's disease and ulcerative colitis as compared with the controls. A potential explanation for the identified metabolites is that they were a consequence of malabsorption caused by the inflammation.

As SIBO has been shown to result in microscopic mucosal inflammation, it is plausible to consider that overall differences in the metabolic profiles of SIBO patients and controls are likely to be found (Riordan et al. 2012; Riordan, McIver, Thomas, et al. 1997a; Haboubi et al. 1991). Also, as it is increasingly believed that a dysbiosis of the gut microbiota is involved in inflammatory bowel disease, either in initiating it or in maintaining it, and as SIBO is also related to dysbiosis, it may be that following the successful application of metabolomics in inflammatory bowel disease, it will also prove relevant in SIBO (Marchesi et al. 2007; Sartor 1997).

Characterisation of the microbial content of the small bowel is a concept that may prove useful in the identification of biomarkers and prognostic factors for SIBO, which might enhance the clinical diagnosis of it. The advantages of these techniques are manifold: rapid, non-invasive and requiring minimal pre-analysis sample preparation.

1.3.2 Research Needs for Small Intestinal Bacterial Overgrowth

Small intestinal bacterial overgrowth is a significant clinical problem, not just in oncology, but also across the field of gastroenterology. It is difficult to diagnose it accurately and there are no optimal therapeutic options. Given the lack of a gold-standard test for SIBO, the concept of discovering novel biomarkers in biofluids using metabolomics technology is attractive. Although, metabolomics technology in the setting of SIBO detection has not yet been investigated, it has the potential to be superior to the currently available diagnostic methods for SIBO and so warrants investigation in this setting.

1.4 Thesis Statement and Research Hypotheses

1.4.1 Research Statement

The nature of OG cancer and the multimodal approach used in its radical treatment puts patients at an increased risk of developing persistent GI symptoms and/or malnutrition. This thesis proposes to establish if there is a persistence of symptoms and malnutrition one year following diagnosis. It is also likely that GI symptoms co-exist with malnutrition rather than occurring in isolation. However, to date the relationship between these two variables has not been elucidated in this patient group. This thesis aims to provide an insight into this relationship. With regard to nutritional screening, there is a need to ensure that the screening tools used in patients with OG cancer are sensitive and specific. The MUST is the most commonly used screening tool in the UK, but has not yet been validated in the OG cancer setting. This thesis aims to validate the tool against an accepted standard (PG-SGA). Finally, due to the poor performance of available diagnostic tests for SIBO, there is a need to pursue new technologies that will improve the ability to detect SIBO reliably. This thesis purposes to investigate the potential of metabolomics technology in the setting of SIBO diagnosis.

1.4.2 Research Hypotheses

1. Disease processes and/or radical treatment result in the persistence or development of moderate-severe GI symptoms at 12 months in OG cancer patients

2. Disease processes and/or radical treatment result in the persistence or development of malnutrition at 12 months in OG cancer patients

3. There is a positive association between GI symptom scores (higher score equals worse GI symptoms) and nutritional status scores (higher score equals worse nutritional status) at diagnosis, during the acute phase of management (3 months) and chronically (12 months) in OG cancer patients

4. The MUST has an acceptable sensitivity and specificity (≥ 70% for both) in the OG oncology setting, by comparison with PG-SGA

5. In patients previously or currently being treated for cancer, qualitative and quantitative analyses of metabolites in urine will indicate the presence or absence of SIBO

Chapter 2

Research Methods

This chapter provides an analysis of the key methods used in this thesis, in the GI and nutritional status study (Chapter 3), MUST validation study (Chapter 4) and the SIBO study (Chapter 5). It provides a rationale for the selection of the methods, including a critical discussion of the literature in the area, as well as a detailed description of the actual method used. The methods include the assessment of GI symptoms, nutritional status, dietary intake and SIBO.

2.1 Gastrointestinal Symptom Assessment

For Chapters 3 and 5, a GI symptom assessment tool was needed. The literature on GI symptom tools was searched, with numerous tools identified that measure GI symptoms in patients with cancer and disorders of the GI tract.

Many scales have been used to measure GI symptoms in the oncology setting, most of which are toxicity scales developed to assess acute symptoms in clinical practice. A number of measures have been based on the graded toxicity scales developed by the World Health Organisation, the National Cancer Institute and US oncology groups (e.g. Radiation Therapy Oncology Group, ECOG, Southwest Oncology Group) (McNulty 1999). These scales adopt the traditional use of clinician ratings of the presence of changes in tissues on the grounds that this is more objective than assessments based on patient reports. The tools refer to toxicity of the following systems/processes: immunology, blood/bone marrow, coagulation, dermatology, haematology, hepatic, auditory, cardiovascular, endocrine, GI, musculoskeletal, lymphatic, metabolic, neurology, visual, pain, pulmonary, renal and reproductive. With regard to the GI system, symptoms assessed by the scales include anorexia, nausea, vomiting, diarrhoea, constipation and mucositis. They do not, however, include any measure of symptom burden; that is, the patient's experience of symptoms and the ways in which these impact on everyday functioning and QoL.

There are accurate and valid tools to assess QoL in oncology, including the FACT-G and the EORTC QLQ-C30. Although the QoL tools in oncology have supplementary site-specific

modules that can focus on specific regions of the GI tract (e.g. oesophagus or stomach), these only include a limited assessment of actual GI symptoms (Darling et al. 2006; Eremenco et al. 2004; Blazeby et al. 2004; Blazeby et al. 2003).

In addition, there are some dysphagia only scores including a five-point swallow score developed in the 1980's by O'Rourke et al. to assess swallowing performance after radiation therapy for carcinoma of the oesophagus (O'Rourke et al. 1988). This tool has since been used in other oesophageal cancer research protocols (Kassam et al. 2008; Coia et al. 1993). Another five-point dysphagia grading scale was developed and used for assessment of the swallow function in OG cancer patients (Ogilvie et al. 1982; Homs et al. 2004). However, these systems only capture the severity of dysphagia, which has been shown to be absent in 70% of patients with early gastric cancers (Allum et al. 2011). Thus, it would not be appropriate to use a dysphagia-only tool, as it would not provide a complete picture of the range of troublesome GI symptoms experienced by patients with OG cancer. Comparably, the four-point Visick scale has historically been used in patients following gastrectomy to record the degree of GI dumping (Visick 1948). Non-surgical patients are unlikely to be affected by dumping syndrome and so this tool is not ideal for patients undergoing chemoradiation without surgery. Also, this tool does not detect other types of GI symptoms experienced and has never been validated.

The Eating Dysfunction Scale was developed by Svedlund et al. in the late-1990's (Svedlund et al. 1999). It is a specific single-site scale including symptoms associated with eating in patients who have had a gastrectomy. However, as with the dysphagia and dumping scales, it would be inappropriate to use a tool that was designed to assess dysfunction in only a sub-group of the overall OG cancer cohort being studied (i.e. the gastrectomy patients).

There are no validated questionnaires specifically designed to measure the frequency, severity and burden of GI symptoms in (a) OG cancer patients acutely and chronically and (b) a mixed group of oncology patients with GI effects of treatment. In view of the absence of oncology-

specific tools that allow the measurement of a broad range of upper- and lower-GI symptoms, the most widely used GI symptom tool in the literature was employed- the GSRS.

2.1.1 Gastrointestinal Symptom Rating Scale

The GSRS is a questionnaire developed in Sweden in 1984 to measure GI symptoms important to patients with general GI complaints and has been validated in previous studies (Svedlund et al. 1988; Kulich et al. 1998; Dimenäs et al. 1993). The results of the GSRS have been shown to correlate with QoL, though the GSRS is not a QoL instrument (Dimenäs et al. 1995; Dimenäs et al. 1993; Wiklund 1995). The instrument has been used in many areas of GI research, for instance in gastro-oesophageal reflux disease, peptic ulcer disease, GI surgery (e.g. pancreatectomy), coeliac disease, chronic intestinal pseudoobstruction, chronic non-specific abdominal complaints and IBS (Kulich et al. 1998; Dimenäs et al. 1995; Dimenäs et al. 1993; Wiklund 1995; Rashid & Velanovich 2011; Iwarzon et al. 2009; Lohiniemi et al. 2000; van den Heuvel-Janssen et al. 2006; Lönroth 2000). It has also shown promise in the GI symptom assessment of patients with GI and extra-GI cancers (Liedman et al. 2001; Namikawa et al. 2011; Russo et al. 2013; Olsson et al. 2007). In a cross-sectional study assessing long term (> six months) nutritional status and QoL following upper-GI surgery for cancer, Carey et al used the GSRS tool to assess GI symptoms (2011). Similarly, Kono et al. used the tool to assess QoL in patients with jejunal pouch reconstruction following total gastrectomy (2003).

It assesses five domains (15 symptoms in total) that have been identified as important to GI function: reflux syndrome (heartburn and acid regurgitation); acute pain syndrome (abdominal pain, hunger pains and nausea); indigestion syndrome (borborygmus, abdominal distension, eructation and increased flatus); diarrhoea syndrome (diarrhoea, loose stools and urgent need to defaecate); constipation syndrome (constipation, hard stools and feeling of incomplete evacuation). The tool requires the patient to rate the severity of their symptoms over the past one or two weeks.

A score of zero indicates the symptom is absent or negligible and that negative social effects are absent. A score of one (mild) indicates that the symptom is noticeable but that negative social effects are absent. A score of two (moderate) indicates that the symptom has a negative physiological impact and a noticeable impact on their social performance. A score of three (severe) indicates that the patient experiences great social and activity-related impairment as a result of negative physiological symptoms. The questionnaire can be administered in either self-report or interview format.

The GSRS was chosen for use as a template in this thesis for a number of reasons. Firstly, its reliability and validity are well-documented (Dimenäs et al. 1995) and normal values for a general population are available (Dimenäs et al. 1996). The internal consistency reliabilities of the five dimensions of the tool (i.e. the extent to which the items within each dimension are interrelated) ranged from 0.6 to 0.85 in patients with duodenal ulcer and from 0.61 to 0.83 in patients with gastro-oesophageal reflux disease (Kulich et al. 1998; Dimenäs et al. 1995). In patients with reflux and dyspepsia, the test-retest reliability of the GSRS dimensions ranged from 0.36-0.75 with a generally low test-retest reliability in the abdominal pain domain (Kulich et al. 1998). This is likely due to the domain containing only two items, as well as the complexity of measuring this symptom. In European patient populations, the GSRS has acceptable construct validity and responsiveness (Dimenäs et al. 1993; Dimenäs et al. 1995; Glise et al. 1995).

Although, the tool has not been validated in oncology, it assesses a broad range of both upper- and lower-GI symptoms likely to be of significance in the oncology setting and the questionnaire has been used to good effect when measuring the presence of symptoms in cancer patients following gastrectomy (Svedlund et al. 1999; Hayami et al. 2011; Ichikawa et al. 2012). However, it does not include items to capture postoperative dysfunction after surgery for OG cancer, for example symptoms of dumping syndrome, early satiety and dysphagia. Also, as with any retrospective questionnaire, the systematic error that is recall bias (i.e. the differences in the accuracy or completeness of the recollections retrieved by study participants regarding events or experience from the past) needs to be considered.

For the purpose of this research, modifications to the questionnaire were made for use in Chapters 3 and 5 following a multi-professional discussion. For both studies, the wording of the questionnaire was revised to make the questions more concise and comprehensible to patients. For the questionnaire used in Chapter 3, the tool was used to capture symptoms burden over a longer time frame (four weeks) than the original tool (one or two week). The rational for this was that the longer interval was more likely to reflect the long-term symptomology in the OG cancer cohort. Also the tool was made more disease-specific by removing and adding certain symptoms. The tool was amended by (a) removing the symptoms of eructation and hunger pains as these were not considered to be relevant in this cohort, and (b) adding nine additional symptoms to the tool, which from clinical experience, were considered to be relevant to OG cancer: dysphagia to solids, dysphagia to fluids, odynophagia to solids, odynophagia to fluids, belching, early satiety, regurgitation of fluids, regurgitation of solids and faecal incontinence (Appendix 8.4).

The GSRS used in Chapter 5 was different to the tool in Chapter 3. The original tool was amended by (a) removing the symptoms of eructation and hunger pains and (b) adding 13 extra items relevant to SIBO: dysphagia to solids, dysphagia to fluids, odynophagia to solids, odynophagia to fluids, belching, early satiety, regurgitation of fluids, regurgitation of solids and faecal incontinence (as per Chapter 3); and vomiting, nocturnal defaecation, steatorrhoea and negative change in stool frequency. Of note, the time frame was the same as the original tool i.e. two weeks (Appendix 8.5).

A Bristol Stool Form Scale was also added to determine the stool form when the patient was *'at best'* and *'at worst'* during the four-week and two-week periods captured by the questionnaire in Chapters 3 and 5 respectively (Lewis & Heaton 1997) .

A self-report approach to the GSRS has been used for this research, although the study dietitian was available to provide assistance with the tool if requested. These modified GSRS tools have not been used in other research or clinical situations.

2.2 Nutritional Screening and Nutritional Assessment

2.2.1 Malnutrition Universal Screening Tool

This tool was used in the MUST validation study (Chapter 4). It was developed by a multidisciplinary group, the Malnutrition Advisory Group (MAG), which is a standing committee of BAPEN (Elia 2003) (Appendix 8.6). It was developed, using evidence-based criteria, to detect protein-energy malnutrition and the risk of developing malnutrition in all adults across all health care settings including oncology (Stratton et al. 2004). The current version of the tool is formed of three steps as follow:

(1) BMI using cut-offs in line with recommendations made by a range of national and international organisations (BMI > 20 kg/m^2, score of 0; BMI 18.5-20 kg/m^2, score of 1; BMI < 18.5 kg/m^2, score of 2).

(2) Unintentional weight loss in past three-six months, using cut-off points that reflect practical and approximate boundaries between normal and abnormal intra-individual changes in weight and the likely presence of a treatable underlying condition, which if undetected could produce further weight loss and malnutrition (< 5%, score of 0; 5-10%, score of 1; > 10%, score of 2).

(3) Acute disease effect producing or likely to produce no nutritional intake for > five days. This allows for the effects of acute conditions that result in no dietary intake, resulting in rapid weight loss (if patient meets these criteria, score of 2).

The numbers obtained from the three steps are additive and produce a MUST score, which indicates the risk of malnutrition and suggests an appropriate action to be taken (Table 2-1) (Elia 2003). The advantages and disadvantages of MUST are shown in Table 2-2.

Table 2-1 Malnutrition Universal Screening Tool: scores, risk of malnutrition and actions

MUST score	Overall risk of malnutrition	Action
2 or more	High	**Treat** unless detrimental or no benefit from nutritional support expected e.g. imminent death
1	Medium	**Observe** or treat if approaching high risk or if rapid clinical deterioration anticipated
0	Low	**Routine care** unless major clinical deterioration expected

Table 2-2 Advantages and disadvantages of the Malnutrition Universal Screening Tool

Advantages	*Disadvantages*
• Quick and easy to complete (< 5 minutes) • Has content validity (comprehensiveness of the tool) • Has face validity (issues which are relevant to the purpose of the test) • Has internal consistency (the extent to which the three steps are interrelated) • Has test-retest reliability (the variation in measurements taken by a single person or instrument on the same item and under the same conditions) • Has fair-good to excellent concurrent validity in hospital in- and out-patients (this validity is demonstrated when a test correlates well with a measure that has previously been validated) • Has some predictive validity, e.g. predicting length of hospital stay, mortality and discharge destination of hospital patients • Excellent reproducibility when different observers assess the same patients	• Unintentional weight loss is a semi-objective criterion, that relies on a patient's ability to remember their weight history • Mathematical errors can occur during the calculation of percentage weight loss • The acute disease effect score is not an objective measure and there is no comprehensive list of acute conditions/ diseases accompanying the tool • No symptom assessment • Continued education of users required

This screening tool has not been well validated for use in the OG cancer setting, and therefore, Chapter 4 will focus on measuring its sensitivity and specificity in OG cancer patients.

2.2.2 Nutritional Assessment: Patient Generated Subjective Global Assessment

Patient Generated Subjective Global Assessment consists of two sections: a patient-completed component and a clinician component (e.g. physician, nurse or dietitian) (Appendix 8.3). The

patient-completed component has four parts (weight loss, nutrition impact symptoms, nutritional intake and functional capacity) and is completed using a check box format. The clinician is required to complete the remainder of the form (diagnosis, age and metabolic stress), conduct a physical examination assessing fat and muscle stores and fluid status and perform a global assessment of nutritional status. It produces a (a) subjective global rating (i.e. SGA) and (b) PG-SGA total score and although they are related, they are independent assessment and triage systems respectively. The subjective global rating categories are consistent with the three categories from the SGA tool:

SGA A	Well-nourished
SGA B	Moderately/suspected malnourished
SGA C	Severely malnourished

There are eight domains in the tool that contribute to the score as follows: weight loss; food intake; nutrition impact symptoms; activities and function; disease and its relation to nutrition requirements; metabolic demand; nutrition-related physical examination and anthropometric assessment. Some of the items are additive, whereas others use the highest score attained. Typical total scores range from 0-35 (maximum score= 49), with scores enabling subtle changes in nutritional status to be identified over a period of two-four weeks. Nutritional triage recommendations using the PG-SGA total score are:

Score 0-1	No intervention required at this time
Score 2-3	Patient and family education with pharmacological intervention and/or laboratory values as appropriate
Score 4-8	Requires intervention by dietitian in conjunction with nurse or physician as appropriate
Score ≥ 9	Indicates a critical need for improved symptom management and/or nutrient intervention options

The PG-SGA was selected for use in this thesis as it has been shown to have a sensitivity of 98% and a specificity of 82% when compared to the SGA (Bauer et al. 2002). It remains the

only validated and specific tool for a thorough nutritional assessment in oncology and as such is used to validate screening tools as referred to in Section 1.2.1. In addition, it is suitable for use as an outcome measure in clinical practice and is associated with QoL in ambulatory patients receiving radiotherapy to the head, neck, abdominal or rectal area and also in a mixed group of patients having chemotherapy (Vergara et al. 2013; Lis et al. 2012).

Although PG-SGA is a relatively easy tool to administer, it does require a trained practitioner for its use. Inter-observer agreement was found in 90% of cases when both a dietitian and doctor performed the assessment using PG-SGA on the same patient (Persson et al. 1999). Some of the advantages and disadvantages of the tool are described in Table 2-3.

Table 2-3 Advantages and disadvantages of the Patient Generated Subjective Global Assessment

Advantages	***Disadvantages***
• Validated in oncology setting • Identifies treatable nutrition impact symptoms • Parameters are weighted based on their nutritional impact • Patient/family participation increases acceptance • Simplifies data collection • User friendly; tables and worksheets included on reverse of form • Serial measures can identify subtle changes in nutritional status • Objective measure to demonstrate the outcome of nutrition intervention • Inclusion of triage recommendations • Gives an indication of quality of life • High rate of interobserver reliability between physicians and dietitians	• Requires more training than other tools • Scoring system can be confusing • Perception of additional workload • Patient generated component relies on patient literacy and recall ability • Physical examination may be resisted by patient and/or healthcare professional • Time intensive

2.3 Dietary Assessment Methods

For the GI and nutritional status study (Chapter 3), a method of assessing habitual dietary intake in OG cancer patients was required. Four dietary assessment methods were considered for use in the study; weighed food diaries, estimated food diaries, the 24-hour recall and the food frequency questionnaire (FFQ). Table 2-4 summarises the general advantages and disadvantages associated with these methods.

Table 2-4 Advantages and disadvantages of dietary assessment methods commonly used to assess food and nutrient intakes

Weighed Food Diaries (usually 3, 5 or 7 days)	
Advantages	***Disadvantages***
• 7-day diaries considered the gold-standard dietary assessment method • Precision of portion sizes • Suitable to capture foods eaten on a regular basis • Estimates for energy, nutrients, foods and food groups have been shown to be excellent • Food/drink recorded at point of consumption and does not rely on individual memory and recall • Provides a detailed description of all foods consumed • Open-ended • 3-10 days is generally sufficient to accurately assess energy and macronutrient intakes	• Costly in staff time and equipment • Labour intensive for participants and researchers • Subjects need to be well-motivated • Misreporting is common • Compliance can be an issue • Subjects might alter actual intake to make it easier to record (e.g. less cooking from scratch, not eating out) • Requires literacy and numeracy skills • Unrepresentative of usual intake if only a few days assessed • Reliability decreases over time due to respondent fatigue • Inappropriate for assessment of past diet • Up to 50 days may be required to assess intake of nutrients where inter-diurnal variation of intake is large

Estimated Food Diaries (usually 3 or 7 days)	
Advantages	***Disadvantages***
• Facilitates collection of information on quantities of foods consumed • Provide useful information on patterns of food used over time, and combinations of foods consumed • An individual's daily intake of energy and nutrients can be calculated • No complex calculations required by subjects in order to fit their food intakes into pre-ordained categories • Lower respondent burden than weighed food diaries	• Relatively high subject burden • Requirement to keep food records for long periods decreases the reliability • Errors can arise in the conversion of estimated to actual food weights • Estimation of portion sizes • Response bias (subject may provide incomplete or false information) • Observation bias may be an issue (respondents may change their intake during the diary period) • Expensive due to data entry costs
24-Hour Recall	
Advantages	***Disadvantages***
• Low respondent burden • Suitable for large scale surveys • Can be administered by telephone or web-based application • Interview relatively quick • Literacy not required • Applicable for a broad population of different ethnicities	• Estimation of portion sizes required • Single observation is seldom representative of habitual intake • Bias in recording *'good/bad'* foods • Memory dependent • Possibility of recall bias (individual may selectively recall food items) • Expensive due to high interview burden but telephone recall can reduce cost • Repeat 24-hour recalls increase time and cost of analysis • Under-reporting is common

	• Reported intakes tend to differ between week days and weekend days • Difficulty in capturing foods eaten rarely or occasionally • Results may vary if a typical day's intake is described rather than intake for the previous 24 hours
Food Frequency Questionnaire	
Advantages	***Disadvantages***
• Suitable for large scale surveys • Useful for ranking subjects to establish low, medium and high consumers • May be self-administered, reducing staff time and effort • Questionnaires may be pre-coded and machine readable • Low respondent burden, quick to complete • Can be posted • Cheap to use due to rapid data entry • Low subject motivation required • Good literacy and numeracy skills not required • Good cooperation from respondents reported • Good reproducibility • Can assess current or past intake • Short versions can focus on specific nutrients with fewer food sources	• List of foods/food groups must be adapted to local population • High level of aggregation of single foods into food groups are required to limit the length of the questionnaire • Relies heavily on memory recall • Assuming a standard portion weight for each food/food group is likely to introduce substantial error • Estimation of portion sizes often difficult (though use of food atlases, household measures etc. may help with this) • Possible over-reporting of healthy foods • Conceptualisation skills needed to estimate frequencies of consumption of certain foods • Observer bias is possible if it is interviewer administered • More accurate if regular eating habits • Quantification of intakes is not as accurate as with recalls or records • Little detail of food characteristics e.g. cooking method, food combinations • Needs to be updated if changes in commonly eaten foods by population

2.3.1 Food Frequency Method

Based upon this critique of the dietary assessment methods, it was decided to use a FFQ in the current thesis because weighed and estimated food diaries were not a realistic option due to the high respondent burden involved, especially in patients with a new diagnosis of OG cancer who are already facing considerable emotional and psychological challenges. Also, the original intention to recruit a larger number of patients would have meant excessive data entry that was not feasible within the scope of this PhD. The FFQ method is the most practical and economical method for the collection of comprehensive dietary data. Dietary intakes of individuals undergoing cancer treatment can vary greatly from day-to-day and week-to-week, due to an altered daily treatment schedule and the GI side-effects of therapies and disease. Therefore, capturing habitual dietary intake using a FFQ would appear to be a more rational approach for this group.

The majority of validated FFQs have been designed to be used by the general population such as that used in the Whitehall II study, which investigated the importance of social class for health (Brunner et al. 2001). A smaller number have been specifically designed for use in populations with or at risk of a particular disease e.g. osteoporosis, cardiovascular disease and prostate cancer (Cade et al. 2004; Patton et al. 1998; Cox et al. 2000; Bairati et al. 1998). There are many tools, which have been validated to assess the associations between the intake of certain foods and beverages and the incidence of certain cancers including OG cancer (Brown et al. 1998; Galanis et al. 1998). However, there are no validated FFQs that assess dietary intake in individuals with an existing cancer diagnosis.

The food frequency approach asks respondents to report their usual frequency of consumption of each food from a list of foods for a specific period. Only information on frequency (and sometimes also quantity) of a list of foods is collected, with little detail on other characteristics of the foods as eaten, such as the methods of cooking or the combinations of foods in meals. The term '*semi-quantitative FFQ*' is used to indicate a general FFQ that allows for a limited

quantification of serving size. Complete FFQs typically must contain 100 or more food items to capture the range of foods contributing to the many different nutrients in the diet.

2.3.2 European Prospective Investigation into Cancer in Norfolk Food Frequency Questionnaire

The dietary assessment tool of choice for Chapter 3 was the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) FFQ (version 6, Appendix 8.7). This is a semi-quantitative FFQ validated for assessing habitual dietary intake for the previous 12 months in the EPIC-Norfolk population (Bingham 1997; McKeown et al. 2001). This FFQ was based on that used in the US Nurses' Health Study (Willett et al. 1985; Willett et al. 1988). The lists of foods were altered by changing American food names to their British equivalent and by using National Food Survey data to identify additional foods that were important sources of nutrients in average British diets. Therefore, its food lists and portion sizes are representative of an adult population likely to have established eating habits and following a traditional British diet.

In Part 1, the EPIC-FFQ contains a list of 130 foods items, followed by a multiple response grid to record the frequency of consumption over the previous 12 months, using one of nine categories ranging from '*never/less than once a month'* to '*more than six times per day'*. Medium servings or units were specified (pints, slices, teaspoons, etc.) for each food item. In Part 2, a number of supplementary questions are included regarding the type of breakfast cereals and fats most often used and the amount of fat usually eaten on meat. A further question on milk is also found in Part 2, requesting information on the type and quantity of milk consumed.

There are two studies that have assessed the accuracy of this FFQ (McKeown et al. 2001; Bingham 1997), both of which have been validated against independent biomarkers of intake and food records. In the study by McKeown et al., the mean reproducibility of the FFQ was moderate to high, with correlation coefficients between the FFQ and an estimated 7-day food diary of 0.64 in men and 0.74 in women. The most notable discrepancies in the reproducibility of

nutrient intake between men and women, as expressed by the crude correlation coefficient, were for protein (0.57 and 0.70, respectively), β-carotene (0.48 and 0.78), non-starch polysaccharideriderides (0.58 and 0.82), and potassium (0.60 and 0.76). The superior ability of a 7-day food diary to document food intake was confirmed in this study.

In the study by Bingham, the reproducibility of the EPIC-FFQ was poorer, with correlation coefficients between the FFQ and a 16-day weighed record ranging from 0.39 to 0.57 (1997). The FFQ overestimated most nutrients when compared with weighed records e.g. daily milk consumption (150 g greater), cheese (15 g greater), and coffee (160 g greater), which largely accounted for the significant differences in energy, fat, protein, potassium, calcium and sugars found between the two methods. In the individuals who completed four different dietary assessment methods, the correlation between urine nitrogen and dietary nitrogen using weighed records was 0.83, using the diary was 0.67, using the FFQ was 0.30, and using the 24-hour recall was 0.12.

In the GI and nutritional status study (Chapter 3), patients were requested to complete the FFQ for intake over the past one month (rather than over the past 12 months) so as to fit with the study design (three study visits in 12 months). No other aspect of the FFQ was adapted. Additional validation studies are recommended when a previously validated instrument is used under new conditions as even subtle changes in its design may affect its performance (Willett 1994). Ideally, this FFQ would have been validated for use over this new time period and in this OG cancer patient group. However, such validation was not within the scope of this thesis, and therefore the reliability of the tool in an acute OG cancer cohort is unknown.

However, despite the limitations of the EPIC-FFQ dietary assessment approach, the benefits of using it in this patient group must be noted. The EPIC-FFQ has a low-respondent burden and is quick to complete, which is important in a patient group with high levels of anxiety, depression and fatigue. Research has shown that 39% and 17% of newly diagnosed GI cancer patients have sub-clinical and clinical anxiety respectively, while 28% and 11% have sub-clinical and

clinical depression respectively (Linden et al. 2012). Also, 28% of GI cancer patients are severely fatigued at the pre-treatment stage (Goedendorp et al. 2008). Given the reported levels of anxiety, depression and fatigue in this group, it was felt that the dietary assessment method chosen would need to be quick and easy to complete. Otherwise, patient recruitment into the study may have been negatively affected. Likewise, the tool can be completed during a study visit (rather than at home), meaning that the patient doesn't need high levels of motivation to complete it and so compliance and acceptability should be improved.

2.4 Diagnostic Tests for Small Intestinal Bacterial Overgrowth

As discussed in Section 1.3.1, all of the commonly used methods for detecting SIBO have inherent limitations. For the purpose of the GI and nutritional status study (Chapter 3), the non-invasive GHMBT was used. For the SIBO study (Chapter 5), both the GHMBT and endoscopic aspiration and culture technique were used. The addition of the direct approach to the GHMBT has been shown to identify a further 16% of patients that would otherwise have been missed (unpublished local data).

2.4.1 Glucose Hydrogen Methane Breath Testing

The GHMBT is a simple, non-invasive indirect test to detect H_2 and methane (CH_4) produced in the GI tract. These gases are primarily produced by the bacterial fermentation of carbohydrates (in this case glucose), so when either of these gases appear in expired air, it is usually a signal that the ingested glucose has been exposed to considerable numbers of bacteria, permitting such fermentation to take place (Levitt 1969).

The GHMBT is based on the physiological observation that healthy, fasting humans at rest do not produce H_2 or CH_4 gas. These gases are only generated during the anaerobic metabolism of nutrients in the gut by bacteria. Therefore, if one or both gases are excreted in exhaled air, they must originate from anaerobic bacteria (Levitt & Ingelfinger 1968; Levitt 1969). If gases are generated in the bowel, it is possible to measure them in exhaled air because they enter the bloodstream by diffusing across enterocytes and are then transported to the lungs, where they

cross the capillary membranes and are excreted during exhalation. Bacteria are ordinarily not present in significant numbers in the small bowel. Therefore, in an individual with SIBO, the generation of these gases will result in their reabsorption into the blood stream from the site of their metabolism by the bacteria.

The measurement of CH_4 excretion while testing for SIBO derives from the fact that CH_4 production is critical for intraluminal H_2 consumption (Strocchi & Levitt 1992). The production of one molecule of CH_4 requires two molecules of H_2 and so it is possible to reduce the total volume of intraluminal H_2 if it is being used for CH_4 production. Therefore, if H_2 is metabolised to produce CH_4, this reduces the amount excreted, which will reduce the breath H_2 peak and could lead to false negative results. Therefore, if H_2 excretion alone is considered as the marker of ongoing small bowel fermentation, any mechanism that reduces H_2 excretion may make the test less accurate.

Thus, breath CH_4 excretion represents an important additional target for an intestinal gas breath excretion measurement in the subgroup of H_2 non-producers, thus enhancing the test accuracy (Corazza et al. 1994). This subgroup could consist of between 2% and 43% of all individuals (Cloarec et al. 1990; Saltzberg et al. 1988; Read et al. 1985; Joseph & Rosenberg 1988; Gilat et al. 1978; Bjorneklett & Jenssen 1982; Flatz et al. 1985). This is in line with findings from our group: a retrospective study (n= 435) demonstrated that the addition of CH_4 to the H_2 breath test identified an additional 20% of patients with SIBO (unpublished data). The predominant methanogen in humans is *Methanobrevibacter smithii* and others include *Staphylococcus aureus*, *Streptococcus viridans*, Enterococci sp., Serratia sp. and Pseudomonas sp.

The GHMBT test consists of the oral administration of a predetermined dose of glucose, with subsequent collection of alveolar breath samples (an air sample that is the last portion of a prolonged, uninterrupted exhalation). Small intestinal bacterial overgrowth is diagnosed by measuring the early appearance of H_2 and/or CH_4 gases following the challenge dose, usually within two hours (Hamilton 1998).

Evidence-based standards concerning the optimal test substrate concentration, the H_2 and CH_4 cut-off levels for test positivity, the frequency of breath sample measurement and the period over which measurements should be taken are lacking and are the topic of ongoing discussion (Romagnuolo et al. 2002; Simren & Stotzer 2006; Ghoshal et al. 2006). However, GHMBT validation studies are divided into two main types based on the substrate dose administered and the test duration: 50 g for 120 minutes and 75-100 g for 180 minutes (Gasbarrini et al. 2009).

The baseline (basal) sample typically has 0-10 parts per million (ppm) of H_2 and 0-7 ppm of CH_4. Ordinarily, basal values of H_2 over 20 ppm are suspicious of SIBO and values between 10 and 20 ppm suggest incomplete fasting for the 12-hour period before the test or the ingestion of foods containing non-absorbed carbohydrates during the day before the test, with the large bowel being the source of the elevated levels (see below) (Hamilton 1998). The samples of expired air are generally collected every 15-20 minutes, while the most frequently used cut-off values for test positivity are: a rise of 10-12 ppm for H_2 and a rise of 6-12 ppm for CH_4 (Gasbarrini et al. 2009). If bacteria are present in the proximal small bowel, H_2 and CH_4 will usually reach the cut-off levels within 20-60 minutes. However, by measuring H_2 for at least three hours for persistently negative tests assures that delayed gastric emptying did not cause a false negative result.

The GHMBT was adopted for use in this thesis because the measurement of CH_4 is likely to improve the test's sensitivity and specificity further (Gasbarrini et al. 2009). Besides being accurate, the GHMBT has other advantages such as non-invasiveness, lack of toxicity, low cost of substrates and easy accessibility in clinical practice.

There are limitations associated with this testing technique including the lack of consensus for a definition of a positive test. Also, a diet low in non-absorbed carbohydrates is advised for the 24 hours prior to the test, increasing the patient burden. It has been shown that a diet low in non-absorbed carbohydrates gives significantly lower basal breath H_2 concentrations, which

facilitates the interpretation of the test results, since changes in H_2 concentrations following glucose challenge can be more easily detected if high *'background noise'* is avoided (Brummer et al. 1985). Breath concentrations of CH_4 appear to be less affected by the ingestion of non-absorbed carbohydrates than are H_2 concentrations (Le Marchand et al. 1992). However, CH_4 responds to disaccharides which escape digestion in the small bowel and hence, the avoidance of disaccharide-containing foods such as dairy products is encouraged during the 24 hours preceding the test (Hamilton 1998). However, as yet there are no published data on the effect of breakfast consumption on breath H_2 or CH_4 levels.

There are other guidelines that patients should adhere to for at least one hour before and for the duration of the test, as non-compliance can affect test accuracy. They should not smoke (as tobacco interferes with H_2 excretion), fall asleep (due to hypoventilation) nor undertake vigorous exercise (due to hyperventilation) (Thompson et al. 1985; Perman et al. 1985). In cases of rapid intestinal transit, glucose may not be completely absorbed in the proximal small bowel (as normal) but rather it quickly reaches the large bowel causing a false positive result (Sellin & Hart 1992). A false positive test result could also occur if bacterial fermentation by oropharyngeal bacteria is not minimised by patients brushing their teeth and/or using mouthwash before the test (Thompson et al. 1985).

2.4.1.1 Equipment and Substrate for the Glucose Hydrogen Methane Breath Test

Glucose hydrogen methane breath tests undertaken as part of Chapters 3 and 5 were performed using a Quintron BreathTracker™ DP Microanalyzer. This is a stand-alone analyser that measures both H_2 and CH_4 simultaneously in an alveolar breath sample (Figure 2-1 a). The machine was calibrated daily prior to any study measurements being taken, as per instructions in the machine manual.

A disposable collection system, the AlveoSampler system is used to collect alveolar air in a standard syringe for immediate analysis. The use of this device eliminates the danger of inter-patient cross-infection and removes the need to clean and sterilise the equipment. Each kit

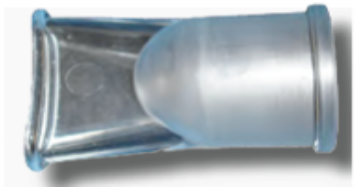
contains all the necessary supplies required for sample collection (a mouthpiece, a vented polyethylene bag, a syringe and a stopcock) (Figure 2-1 b and c). The substrate used for the test was glucose, a monosaccharide that is completely absorbed in the proximal small bowel. The test dose was 50 g for patients weighing < 50 kg and 75 g for those weighing > 50 kg.

For samples that could not be analysed in real-time, Quintron's Sample Holding Bags were used (Figure 2-1 d). These are small foil-laminated bags with a capacity of 250 ml designed to safely hold samples until the analyses can be completed. After collection, the alveolar air was passed through a drying agent and transferred to a holding bag using the syringe. Each bag holds a single breath sample for up to two weeks with minimal loss of sample integrity. A stopcock was put into the Luer port on the bag prior to transferring the sample to minimise the loss or alteration of the sample. These bags did not come in contact with patients directly and were re-used multiple times having been maintained according to the manufacturer's instructions.

The machine uses the basic principles of gas chromatography, which separates the gas components. Room air was used as the carrier gas, which is pumped through the system where the H_2 and CH_4 were separated from each other and from all other reducing gases and were then carried sequentially past a solid-state sensor. The signals were processed and the sample concentrations were displayed in ppm, within a short analytical time of 50 seconds or less.

(a)

(b)



(c)

(d)

Figure 2-1 Glucose hydrogen methane breath test equipment: (a) Quintron BreathTracker™ DP Microanalyzer, (b) vented polyethylene bag, syringe and stopcock, (c) mouthpiece, (d) Sample Holding Bag

2.4.1.2 Test Preparation for the Glucose Hydrogen Methane Breath Test

Patients with insulin-dependent diabetes mellitus were excluded from undergoing a GHMBT. As the test involves a 12-hour fasting period, resulting in the risk of hypoglycaemia, it is not an appropriate test for these patients. Prior to the GHMBT, all other patients received an information booklet, describing the test and providing detailed preparation guidelines for the day before and the day of the test (Appendix 8.8). Patients were requested to avoid non-absorbed carbohydrates for 24 hours and to fast for 12 hours before the GHMBT, only drinking water during this time. Patients were encouraged to brush their teeth and/or use mouthwash on the morning of the test.

Testing was performed by the study dietitian at RM's Endoscopy Unit (Fulham Road, London) or at the Department of Rehabilitation at the hospital's Sutton site. Whenever possible, testing

took place in the early morning. For at least one hour before the test (and also during it) patients were requested not to undergo vigorous activity, smoke tobacco or fall asleep.

2.4.1.3 Testing Protocol for the Glucose Hydrogen Methane Breath Test

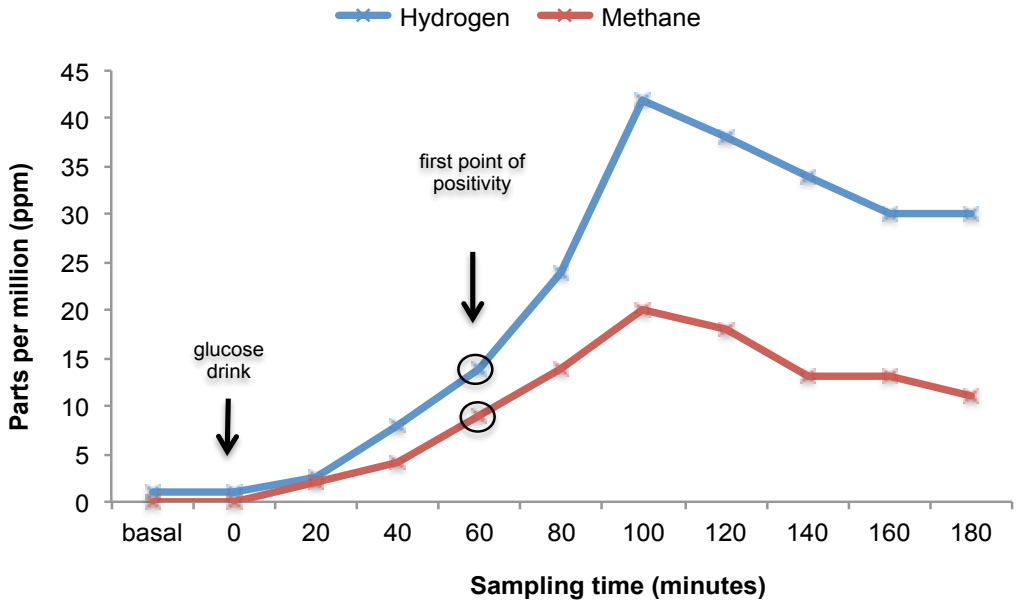
At the beginning of the test, the study dietitian verified that the patient had adhered to the test diet, had fasted for the previous 12 hours, not smoked tobacco, exercised or taken any sugar-containing medication on the morning of the test and had brushed their teeth/used mouthwash before the test. If the patient had incorrectly prepared and the basal H_2 was raised, the GHMBT was not performed and was re-scheduled. The test was commenced with the collection of an initial basal breath sample. Patients were seated for this and all subsequent measurements.

Using the AlveoSampler system, the basal breath sample was collected as follows: the patient took a normal breath and, at the end of inspiration, put the mouthpiece into their mouth, sealed their mouth and exhaled normally through the mouth into the bag. The polyethylene bag had a small vent hole and as it filled with air it was vented allowing exhalation to continue. The patient continued the exhalation until all air from the lungs was respired i.e. adequate dead space volume had been exhaled. At this point, the patient made a signal (by raising a hand) to the study dietitian, who steadily drew into the syringe and retained the 30 ml of end-expiratory air blown through the device (Figure 2-2 a). Until the study dietitian had sealed off the stopcock, the patient kept their mouth tightly sealed around the mouthpiece. The sample was either analysed shortly after collection (Figure 2-2 b), or stored in the syringe for up to four hours. Alternatively the sample was passed into a Sample Holding Bag for analysis at a later time, as described in Section 2.4.1.1.

(a) (b)

Figure 2-2 Glucose hydrogen methane breath test: (a) sample collection, (b) sample analysis

Assuming the basal reading did not indicate a positive test, the test substrate was administered as a glucose solution (i.e. 50 g or 75 g of glucose dissolved in 150 ml of warm water). The solution was consumed orally and the time of ingestion was noted. Breath samples were collected (as for basal sample) every 20 minutes after this point until a positive reading was noted or if readings remained persistently negative, for a total of 180 minutes. During this time, the patient could drink water in moderation but continued to avoid all other beverages, foods and smoking. A positive result was recorded if there was: (a) a fasting H_2 level ≥ 20 ppm, (b) a rise of ≥ 12 ppm H_2 above baseline during the test, (c) a fasting CH_4 level ≥ 10 ppm and/or (d) a rise of ≥ 6 ppm CH_4 above baseline during the test, as per those criteria used by Lisowska et al. (2009). An example of a typical positive test (for both H_2 and CH_4) is shown in Figure 2-3. When a positive result had been recorded, the test was usually discontinued. If the test was persistently negative but was not continued until 180 minutes, it was considered incomplete.



	basal	20	40	60	80	100	120	140	160	180
H_2, ppm	1	3	8	14	24	42	38	34	30	30
CH_4, ppm	0	2	4	9	14	20	18	13	13	11

Figure 2-3 Typical positive glucose hydrogen methane breath test: the test was first positive for hydrogen gas and methane gas at 60 minutes

2.4.2 Endoscopic Aspiration and Culture Technique

For the SIBO study (Chapter 5), a microbiological quantification method was used to detect SIBO. There remains a lack of clarity on the cut-offs that define a positive culture. In the literature, there is great diversity in defining a positive culture with some definitions including the following intestinal bacterial counts:

- Aerobic bacterial counts of more than 10^5 CFU/ml (Khoshini et al. 2008; Ford et al. 2009)
- Anaerobic bacterial counts of more than 10^4 CFU/ml (Khoshini et al. 2008; León-Barúa et al. 1993)
- Growth of any Enterobacteriacae sp. or gas-forming organisms (Henriksson et al. 1993)

- More than 10^3 CFU/ml of anaerobes or more than 10^4 CFU/ml of aerobes excluding *Streptococcus viridans* and Lactobacilli sp. (Farivar et al. 1979)
- More than 10^3 CFU/ml of Enterobacteria sp. or Enterococcus sp. or more than 10^4 CFU/ml of Bifidobacteria sp., Clostridia sp. and Bacteroides sp. belonging to the *B. fragilis* group (Rumessen et al. 1985)

In a systematic review of the diagnostic tests for SIBO, 50 studies used the results of small bowel aspirate culture to determine SIBO. The most commonly cited definition was: 10^5 or more CFU/ml of bacteria or fungi grown from the small bowel aspirate (Khoshini et al. 2008).

This diagnostic approach was used in Chapter 5 in addition to the GHMBT to provide a more robust approach to SIBO diagnosis. The OGD also allows the gastroenterologist to assess the patient for other causes for their GI symptoms including *H. pylori* infection, gastritis or duodenitis, coeliac disease or recurrent/new oncological disease. For positive results following quantification, the microbiology report provides the team with microbial sensitivities, which guide antibiotic treatment and avoids the inappropriate use of antibiotics.

With this endoscopic aspiration approach there are a number of limitations to be considered including its invasiveness and the low, but measurable risk associated with endoscopic intubation (Table 1-11). It is time-consuming and cumbersome, aspirations can be difficult to obtain and insufflation of air into the lumen can prevent accurate sampling. Also, the endoscope may be contaminated by bacteria proximal to the site of sampling. The location and amount of small bowel fluid recovered has been shown to be highly variable (Khoshini et al. 2008). With regard to the location, the general consensus is that aspiration should take place beyond the ligament of Treitz. However, the distribution of SIBO might be patchy, possibly occurring in the distal small bowel or in a difficult-to-access area (e.g. a blind loop) and therefore a single sampling site may not always detect SIBO.

Aspiration-based approaches also suffer from technical difficulties associated with transporting and culturing the aspirate. It places high demands on the quality of laboratory work and has several difficulties including low reproducibility, the need for anaerobic techniques and an inability to identify cultivation-resistant bacteria. Anaerobic techniques are not always readily available in a clinical setting and so false-negative results may occur where overgrowth is caused by obligate anaerobes. Furthermore, culturing reveals only a fraction (estimated at 20%) of microbiota compared with genomic methods (Eckburg et al. 2005).

2.4.2.1 Testing Protocol for Jejunal Aspiration

All procedures were carried out at RM's Endoscopy Unit (Fulham Road site) by one of three gastroenterologists, assisted by trained endoscopy nurses. The microbiological culturing and analysis of the samples was performed at the hospital's Sutton site by a team of microbiologists.

The procedure was performed after the patient had fasted for 12 hours. During the endoscopy procedure, a sterile endoscope was passed through the stomach and into the small bowel. The sample to be cultured was obtained at approximately 25 to 30 cm beyond the pylorus or at the ligament of Treitz (i.e. in the first part of the jejunum). When the desired collection site was chosen, a sterile catheter was inserted through the washing channel of the endoscope. One hundred ml of sterile saline were then vigorously flushed into the jejunum, with care being taken to use minimal air-insufflation, so as to maintain the intraluminal anaerobic environment. The suction was then turned down and after leaving the jejunal secretions and saline to equilibrate for 10-20 seconds, at least 5 ml of jejunal fluid was suctioned through the inner tube and collected in the attached sterile Pennine trap.

The trap was immediately sealed to prevent anaerobic contamination by oxygen in the air. The appearance of the aspirate was recorded i.e. crystal clear, cloudy or bile stained. This information was used to determine how representative the sample was, with bile stained samples being more likely to indicate that the sample has been sitting in the upper small bowel for some time compared with crystal clear samples. The samples were stored in a dedicated

refrigerator maintained between 0 and 4°C before being transferred to the Sutton site for microbiological culture and analysis in sealed polyethylene bags (temperature maintained between 0 and 4°C) between one and 12 hours after the sample collection.

2.4.2.2 Microbiological Quantification of Jejunal Aspirate

Following the one-hour trip to the microbiology laboratory, the jejunal samples were analysed immediately. Each aspirate sample was cultured on (a) ANE neomycin blood agar anaerobically for 48 hours at 37°C (for anaerobic cultures), (b) CPA/CAN bi-plate in air for 24 hours at 37°C (for aerobic cultures), and (c) Sabourauds agar in air for 48 hours at 30°C (for aerobic cultures). All anaerobic manipulations were performed in an anaerobic glove box in an atmosphere of 10% CO_2, 10% H_2 and 80% N_2. A quantitative culturing method was employed in the laboratory to report the growth of fungi, aerobic and anaerobic bacteria following the culturing process. Any bacterial growth > 10^3 CFU/ml was followed up with full identification and sensitivities. The microbiological cut-off values used to determine test positivity in Chapter 5 have previously been used by Choung et al. and are shown in Table 2-5 (Choung et al. 2012).

Table 2-5 Microbiological cut-off levels for jejunal aspirate culturing

Result	Microbial Growth
Negative Aspirate Culture	0 CFU/ml for both aerobic and anaerobic bacteria
Intermediate Aspirate Culture	0 CFU/ml to 10^5 CFU/ml of aerobic bacteria **or** 0 CFU/ml to 10^4 CFU/ml of anaerobic bacteria
Positive Aspirate Culture	> 10^5 CFU/ml of aerobic bacteria **or** > 10^4 CFU/ml of anaerobic bacteria
Abbreviation: CFU/ml, colony-forming unit per millilitre Note: A patient may fulfil more than one criterion for an intermediate or positive aspirate culture	

Chapter 3

Gastrointestinal Symptoms and Nutritional Status in Patients with Oesophagogastric Cancer: A Longitudinal Cohort Study

3.1 Introduction

3.1.1 Rationale

There are numerous studies in patients with OG cancer who have undergone radical treatments with respect to postoperative complications and their disease-free and overall survival at one year (Lee et al. 2014; Kubo et al. 2014; Cunningham et al. 2006; Anderson et al. 2011; Gillham et al. 2008; Coupland, Allum, et al. 2012a). It is well documented that QoL can be compromised during and after treatment for these cancers (Blazeby, C. Metcalfe, et al. 2005a; Scarpa et al. 2011; Courrech Staal et al. 2010). Interestingly, patients' well-being and feelings of recovery have been shown to be dependent on good GI functioning and them returning to a pre-diagnosis nutritional state (Olsson et al. 2010). However, quantitative research assessing GI function and nutritional status during the first year following diagnosis is limited and in particular, there is almost no data on the relationship between these two variables.

Researchers have been interested in assessing the need for artificial nutritional support (i.e. oral nutritional supplements (ONS), EN and parenteral nutrition) amongst patients with OG cancer, especially following surgery. Still, little is known about the nutrient and food group intake of these patients as they progress through radical treatment and following its completion. This knowledge, as well as an understanding of their nutritional status and GI symptoms would provide a better understanding of their needs from a dietary and symptom control perspective.

The oncological treatments received by patients with OG cancer can alter the anatomy of the GI tract, cause dysmotility and hypochlorhydria and impair immune function. As such, these patients have predisposing factors for the development of SIBO. Clinical experience suggests that the GI symptoms reported by patients with OG cancer in the months following treatment are very similar to those described by patients with SIBO. These symptoms include nausea, bloating, abdominal pain and grumbling, diarrhoea and flatulence. However, SIBO as a potential mechanism for the GI symptoms and/or malnutrition in patients with OG cancer has rarely been investigated. If the cause for their ongoing GI symptoms was found to be linked to SIBO, this could have important implications for their management during and after treatment.

3.1.2 Hypotheses

1. Disease processes and/or radical treatment result in the persistence or development of moderate-severe GI symptoms at 12 months in OG cancer patients

2. Disease processes and/or radical treatment result in the persistence or development of malnutrition at 12 months in OG cancer patients

3. There is a positive association between GI symptom scores (higher score equals worse GI symptoms) and nutritional status scores (higher score equals worse nutritional status) at diagnosis, during the acute phase of management (3 months) and chronically (12 months) in OG cancer patients

3.2 Study Objectives and Outcomes

3.2.1 Study Objectives

The primary objective was to measure GI symptoms and nutritional status in patients with OG cancer at three study time points: baseline (pre-treatment), acutely (3 months) and chronically (12 months).

Secondary objectives were as follows:

- To determine if moderate-severe GI symptoms persist or develop between baseline and 12 months
- To describe Bristol Stool Form Scale data at the three study time points
- To determine if malnutrition persists or develops between baseline and 12 months
- To determine the association between (a) the presence of individual GI symptoms and (b) total score from all GI symptoms combined and nutritional status at the three study time points
- To describe the nutrient and food group intake pattern in patients at the three study time points and determine whether estimated requirements are being met

- To report the prevalence of SIBO at baseline and its incidence at 3- and 12 months in a sub-group of patients tested for it

3.2.2 Study Outcomes

The primary outcomes included: individual GSRS scores for 22 symptoms (0-3), GSRS total scores (0-66), PG-SGA total score (0-49) and PG-SGA categories (SGA A, B, C) at the three study time points.

Secondary outcomes included:

- Percentage weight loss in previous three-six months
- Stool types when *'at best'* and *'at worst'*
- Daily intake of energy, macronutrients, fibre, micronutrients and food groups
- A positive GHMBT result

3.3 Study Design, Population and Organisation

3.3.1 Study Design and Population

This was a prospective, observational, longitudinal cohort study in patients with OG cancer undergoing radical treatment. Each patient underwent staging investigations, including an OGD, to obtain biopsies for histopathological confirmation of cancer/pre-malignant condition. Patients were planned for assessment at three visits during the study: at diagnosis before beginning treatment (baseline study visit), three months after commencing treatment (3 month study visit) and 12 months after commencing treatment (12 month study visit).

The study's inclusion criteria were as follows:

- Newly diagnosed with malignant cancer of the oesophagus, GOJ or stomach or pre-malignant condition of these regions (e.g. high-grade dysplasia, Barrett's oesophagus)
- Planned for radical treatment for their OG cancer
- Age ≥ 18 years
- Ability to give written informed consent to participate

The study's exclusion criteria were as follows:

- Recurrent disease
- Intended for palliative treatment
- Patient decision not to undergo radical treatment
- Radical treatment began more than one week before baseline measurements could be performed
- Inability or unwillingness to give informed consent
- Incapacity to comply with the demands of the study
- Inability to adequately understand verbal or written information given in English

For simplicity, from this point onwards the term *'OG cancer'* will be used to encompass this cohort of patients with malignant and pre-malignant diseases of the OG region. Of note, after the commencement of the study, a minor amendment to the study protocol was sought (and approved), which allowed the expansion of the inclusion criteria to include pre-malignant conditions. Therefore, some of the patients screened before this amendment were excluded because their tumour was pre-malignant.

3.3.2 Study Organisation and Responsibilities

The study (CCR 3703) was granted ethical approval by the London-Riverside Research Committee (NHS Brighton and Hove) on 31st October 2011 (Appendix 8.9). The study was conducted in accordance with the ethical requirements of the Declaration of Helsinki (1996) and good clinical practice. The RM's Committee for Clinical Research authorised the study on 16th November 2011. It was approved as a single-centre study, with recruitment taking place at the hospital's Sutton and London sites. The RM's Charitable Trust funded the study.

Overall responsibility for this study rested with the Principal and Chief Investigator, Dr Jervoise Andreyev (Consultant Gastroenterologist in Pelvic Radiation Disease at RM). Co-investigators included Dr Clare Shaw (Consultant Dietitian at RM), Prof Kevin Whelan (Professor of Dietetics at King's College London, KCL) and the thesis author, Ms Eva Grace, (Research Dietitian at RM

and PhD student at KCL). All four investigators were involved in the study's design and progress, while the day-to-day running of the study and data acquisition was the responsibility of Ms Grace.

Mr Amyn Lalji, Database Manager at RM was responsible for the secure protection, preservation and integrity of hard copy data records and electronic data. The data were also entered into the study database by Mr Lalji. Throughout the study, patient data were handled in accordance with the requirements of the Data Protection Act (1998) and in accordance with RM data protection and confidentiality arrangements. All data were treated as strictly confidential and held in a secure location. There were three study statisticians who assisted with statistical work: Ms Karen Thomas and Mr Kjell Pennert at the study design phase and Mr Kabir Mohammed at the database development, data extraction and analysis phases. The RM employs all of these individuals. Monthly research meetings held at RM took place during the course of the study. All co-investigators were invited to attend these meetings, which were a forum to discuss study progress including recruitment trends, ethical issues and withdrawals.

3.4 Methodology

3.4.1 Screening, Inviting and Consenting

Each patient with a new OG cancer diagnosis was discussed at a weekly OG specialist MDT meeting at RM. A treatment plan was established for each individual, which was influenced by histopathological results, past medical history, ECOG performance status, fitness for surgery, social circumstances and other clinical issues driving their management (as described in Section 1.1.1). The decision to proceed with radical treatment was made by this OG specialist MDT at these meetings and the study dietitian screened patients to identify those fulfilling the study's inclusion criteria.

Given the vulnerability of the patient group, the study dietitian liaised with other members of the MDT (e.g. research nurses and clinical nurse specialists) to determine the most appropriate time to approach eligible patients, particularly those who were emotionally fragile. It was hoped

that this would minimise stress for the patients and avoid overwhelming them with information, especially as many were also eligible for participation in other research studies and drug trials at RM. At a suitable time and in a private location, the patient was approached by the study dietitian, often at one of their routine out-patient appointments. The study was described in detail to the patient and its voluntary nature was emphasised. Patients who expressed an interest were provided with written information in the form of a Patient Information Sheet (Appendix 8.10). Permission to contact the patient by telephone (or email if favoured) was obtained and the patient was provided with contact details should they wish to discuss the study further. Each patient was given a minimum of 24 hours to consider participation in the study. Only after this time, if they agreed to participate, could written consent be obtained (Appendix 8.11). After consent was obtained, each patient was registered on the hospital's *'Committee for Clinical Research Protocols for Patients'* electronic system. Patients were free to withdraw at any point, without having to provide a reason and without it affecting their ongoing care or treatment.

The first study visit was arranged, allowing sufficient time for the consent process and baseline measurements to be undertaken. This and the two future study visits were organised for either the London or Sutton site, depending upon the patient's preference. For out-patients, the study visits were set to coincide with their other clinical appointments whenever possible, to avoid unnecessary hospital attendances. When a patient was receiving treatment as an in-patient, they were seen on the ward.

3.4.2 Data Collection and Entry

After enrolling a patient, the study dietitian collected information on a case report form (paper copy) for the three study visits. The data included on the case report form are described in Table 3-1. Details of the questionnaires completed and the GHMBTs performed are discussed in Sections 3.4.2.1, 3.4.2.2, 3.4.2.3 and 3.4.2.4. Study data was entered into a secure RM study database in a timely manner. This helped to avoid cases of lost or missing data. The study dietitian performed checks on 10% of all entered data before statistical analysis was permitted.

Table 3-1 CCR 3703: data collected at the three study visits

Data collected	Study visit			Where collected from
	Baseline	3 months	12 months	
Demographic and clinical information				
Baseline demographics	✓	✗	✗	EPR, patient
Tumour site and histological stage	✓	✗	✗	EPR
ECOG performance status	✓	✗	✗	EPR
Other gastrointestinal diagnoses and presence of ileostomy/ colostomy	✓	✗	✗	EPR, patient
Conditions and medications that may predispose to SIBO	✓	✓	✓	EPR, patient
Nutritional information				
Presence of oesophageal stent	✓	✓	✓	Patient, EPR
Source of nutrition (food, ONS, EN, parenteral)	✓	✓	✓	EPR, patient
Modifying texture of food	✓	✓	✓	Patient
Number of consultations with a clinical dietitian before/during study	✓	✓	✓	EPR
Treatment information				
Intended oncological treatment	✓	✗	✗	EPR
Oncological treatment received	✗	✓	✓	EPR, patient
Treatment completion date	✗	✓	✓	EPR, patient
Questionnaires				
Modified GSRS	✓	✓	✓	Patient
PG-SGA	✓	✓	✓	Patient
Modified EPIC-Norfolk FFQ	✓	✓	✓	Patient
Test for small intestinal bacterial overgrowth				
GHMBT	Refer to Section 3.4.2.4			Patient
Abbreviations: ECOG, Eastern Cooperative Oncology Group; EN, enteral nutrition; EPIC-Norfolk FFQ, European Prospective Investigation into Cancer-Norfolk food frequency questionnaire; EPR, electronic patient record; GHMBT, glucose hydrogen methane breath test; GSRS, Gastrointestinal Symptom Rating Scale; ONS, oral nutritional supplements; PG-SGA, Patient Generated Subjective Global Assessment; SIBO, small intestinal bacterial overgrowth				

3.4.2.1 Modified Gastrointestinal Symptom Rating Scale

As described in Section 2.1.1, the modified GSRS was used to measure the prevalence and severity of 22 upper- and lower-GI symptoms experienced over the previous four weeks (Appendix 8.4). The tool will be referred to as *GSRS* for the remainder of this chapter.

3.4.2.2 Patient Generated Subjective Global Assessment

As discussed in Section 2.2.2, the PG-SGA assessment involved a patient component (Boxes 1-4) and a clinician component (Worksheets 1-5) (Appendix 8.3). The study dietitian was trained

to use the tool by shadowing a PG-SGA trained consultant dietitian (Dr Shaw) as she performed assessments on 12 patients and by watching the PG-SGA training video tape (Davis McCallum & Polisena 2001). In addition, the consultant dietitian and study dietitian both independently performed PG-SGA assessment on six patients and afterwards scorings and categories were compared to ensure homogenous results, thereby confirming that the study dietitian was sufficiently trained in PG-SGA assessment.

The RM's Patient Height and Weight Policy and Procedures document was followed for the measuring and recording of the patients' height and weight (The Royal Marsden NHS Foundation Trust 2014; Dougherty & Lister 2011). The weighing scales used were the Marsden M-120 Column Scales and height was measured using the Marsden HM-200 Telescopic Height Measure. In patients who were unable to stand safely or comfortably, weight was measured using the Marsden M-210 Chair Scales and height was estimated using ulna length, as described in the above RM document. Ulna length is an accurate alternative to measured height in older individuals (Reidlinger et al. 2014). Scales and height measures were positioned in each ward, day unit and out-patient area. All scales and height measures were serviced and calibrated every six months by the equipment manufacturer.

For patients weighed on the standing scales, the calibrated scales were positioned on a level surface and connected to the mains electricity. The patient was asked to remove their shoes and outdoor garments, so that they were wearing light day clothing only. Items in pockets and/or jewellery were removed. It was ensured that the scales recorded zero prior to asking the patient to stand on them. Once on the scales, the patient was asked to remain still and it was checked that they were not supporting any weight on any object e.g. wall, stick or feet on the floor. The study dietitian measured weight to 0.1 kg.

The presence of ascites and/or oedema was noted and where present, an estimated weight was recorded using the BDA handbook (Parenteral and Enteral Nutrition Group of the British Dietetic Association 2011). Weight loss, if applicable over the past one month (or six months)

was determined using the hospital's electronic patient record (EPR) system, if available, or using the patient-reported change in weight.

Standing height was measured with the participants standing barefoot. The patient was asked to stand as upright as possible and look directly ahead. It was ensured that the bottom of the ear lobe and nose were in a horizontal plane. The height bar was then gently lowered, in a horizontal plane, until it touched the top of the patient's head and height was measured to the nearest 0.1 cm.

Patients were requested to wear loose fitting clothes on the day of each study visit to allow the physical examination component of the PG-SGA to be performed. Verbal consent was obtained before the examination was undertaken. To ensure consistency, the study dietitian used the SGA Physical Examination Guidance Sheet developed by Dr Shaw for use in another RM study (Appendix 8.12).

Following the completion of all components of the tool, the PG-SGA total score (0-49) and the subjective global rating (SGA A, B or C) were determined and recorded. Using the nutritional triage recommendations, when a patient had a PG-SGA total score of 2-3 and had not already been referred to a clinical dietitian, they were provided with an Eating Well When You Have Cancer booklet (The Royal Marsden NHS Foundation Trust 2002). This is a resource designed for individuals who are at risk of malnutrition or already malnourished, which is routinely used within the Department of Nutrition and Dietetics at RM. In addition, the patient was provided with the contact details for this department, should they require an appointment before the next study visit. When a patient had a numerical score of ≥ 4 and if there was no previous referral to a clinical dietitian, the patient was offered an out-patient dietetic appointment. If this offer was declined, the patient was given the booklet and relevant contact details should they change their mind.

3.4.2.3 Modified European Prospective Investigation into Cancer in Norfolk Food Frequency Questionnaire

The use of the modified EPIC-Norfolk FFQ (Appendix 8.7) has previously been discussed (Section 2.3.2) and will be referred to as *FFQ* for the remainder of the thesis. At each study visit, if a patient had received any nutrition from food sources over the previous one month, a FFQ was completed. The questionnaire was self-administered but was completed in the presence of the study dietitian, who provided clear instructions and assistance to the patient, if requested. Each questionnaire took 15-20 minutes to complete. For those patients whose sole nutritional source during the previous month was from EN, parenteral nutrition and/or ONS, a FFQ was not completed. In these cases, and in instances where a proportion of an individual's nutritional intake came from these sources, the type and volume of formula/supplements taken per day (on average) was recorded for the previous one month. These details were acquired using the clinical dietetic notes on the EPR system, by liaising with the clinical dietitians and by gathering information on volume and frequency from the patient and nursing staff, as appropriate.

Data entry and analysis of the questionnaires was undertaken by the study dietitian using the Food Frequency Questionnaire European Prospective Investigation into Cancer and Nutrition Tool for Analysis (FETA) software to produce nutrient and food group intake data. Gratitude is extended to the EPIC-Norfolk Study team for the use of the FETA EPIC-FFQ software (Mulligan et al. 2014). They are supported by programme grants from the Medical Research Council United UK (G9502233, G0300128) and Cancer Research UK (C865/A2883).

The software produced nutrient and food group data for the FFQs. Responses to each food item were coded 1-9 from '*never or less than once/month*' (code 1) to '*6+ per day*' (code 9). If a food item had missing frequency data, -9 was entered. Questionnaires were considered incomplete if ten or more food items had missing frequency data and were therefore excluded from analysis (Welch et al. 2005). Answers to the questions in Part 2 were matched and converted into the appropriate nutrient database codes by using milk, cereal and fat reference lists. Daily intake of

macronutrients, micronutrients and food groups were produced by the FETA software (Mulligan et al. 2014).

One drawback of the FETA software is that it does not include data on nutritional composition of ONS or enteral formulas, which are an important source of nutrition in patients with OG cancer. In addition, although patients can add commonly eaten food items not covered by the FFQ to the questionnaire, the FETA software program is not yet capable of computing them (Mulligan et al. 2014). To overcome these issues, the software Dietplan© was used (version 6.70.36, Forestfield Software Ltd, UK). The intake of any ONS, enteral formulas and additional non-standard foodstuffs were entered into the Dietplan software. To allow this analysis to be performed, the following food and product tables were downloaded from the Dietplan 6.70 installation CD: ABT Abbott Nutrition, FRS Fresenius Kabi Ltd, NCC Nutricia Advanced Medical Nutrition, NES Nestle Nutrition and NVT Nestle (ex. Novartis) Medical Nutrition. As with the data from the FETA software, daily intake of macro- and micronutrients were obtained for ONS, enteral formulas and additional non-standard foodstuffs, although Dietplan© does not produce food group data.

Finally, the data from FETA (from the EPIC FFQs) and Dietplan© (from ONS, enteral formulas and additional foods) were combined as appropriate, on an individual basis.

3.4.2.4 Glucose Hydrogen Methane Breath Testing

When the study opened, it was intended that all participants would undergo three GHMBTs- one at each study visit. However, following six months of recruitment, it was apparent that this was not achievable because many eligible patients were refusing to participate because of the requirement to perform the GHMBTs (i.e. ten of the first 25 who declined, declined for this reason). Each GHMBT involves a diet change for 24 hours, a 12-hour fast and the test itself can take three hours to complete. Given that the patients were already spending considerable time undergoing other staging investigations at the hospital (e.g. OGD, laparoscopy,

cardiopulmonary exercise test), an additional test was enough to dissuade many from participating.

Therefore, the study dietitian decided that all eligible patients would continue to be invited to participate with the GHMBT being included as a study measurement. However, if the patient refused to take part because of an unwillingness to undergo the GHMBT, then the patient would be offered the option of completing the other study components outlined above (GSRS, PG-SGA, FFQ). The London-Riverside Research Committee approved this substantial amendment to the study protocol on 27th July 2012 (Appendix 8.13).

For those patients willing and eligible to undertake the GHMBT, the equipment and substrate, the preparation for the test and the test protocol have previously been described in Sections 2.4.1.1, 2.4.1.2 and 2.4.1.3 respectively. Only those individuals, who performed a GHMBT at the baseline visit, were requested to undergo the test at subsequent study visits.

3.4.3 Statistical Methodology

3.4.3.1 Sample Size Calculation

This was an observational study with no group comparisons and no reporting of effect size. Therefore, it was not necessary to power the study.

3.4.3.2 Statistical Analysis Methods

Statistical analyses were conducted using the Statistical Package for the Social Sciences software (SPSS, version 22.0, IBM, USA). In all statistical testing a 2-sided significance level of 5% was used to assess significant difference between paired data or between patient groups. No missing data was replaced. The Komogorov-Smirnov test was used to visually assess GSRS total scores and PG-SGA total scores: both were non-normally distributed. As such, median, range and interquartile ranges (IQR) were used to summarise the data and compare the paired data using non-parametric tests.

For the individual GSRS symptoms, numerical values for each of the 22 symptoms were reported (0= none, 1= mild, 2= moderate, 3= severe). The maximum GSRS total score was, therefore, 66. Summary statistics were reported at each time point using count (percentage) of patients reporting individual symptoms, the median (range) number of symptoms and the median (range) GSRS total score**.** Paired scores comparisons were undertaken using Wilcoxon non-parametric tests to establish any change in GSRS total scores between time points. The *'persistence'* of a GI symptom occurred when a patient reported the symptom as moderate or severe at baseline and also at 12 months, with movement between moderate and severe allowed. The *'development'* of a GI symptom occurred when a patient reported the symptom as none or mild at baseline but as moderate or severe at 12 months. To establish if symptoms persisted or developed at the 12 month point, the number of individuals with moderate-severe symptoms were compared at baseline and 12 months in those patients followed up at the latter time point.

For the Bristol Stool Form Scale, the frequency of stool types when *'at best'* and *'at worst'* were described, with groupings as follows: (a) Types 1 and 2, (b) Types 3, 4 and 5 and (c) Types 6 and 7. These categories were chosen as Types 3, 4 and 5 are generally considered to be in the normal range.

On completion of the PG-SGA, each patient was categorised as follows: well-nourished (SGA A), moderately/suspected malnourished (SGA B), or severely malnourished (SGA C). In addition, a PG-SGA total score was calculated where the lowest (best) score was 0 and the highest (worst) score was 49. Scores for Boxes 1, 2, 3 and 4 (which relate to weight, food intake, symptoms, activities and function) and Worksheets 2, 3, and 4 (which relate to relevant diagnoses, metabolic demand and physical examination) of the tool were reported separately. The median (range and IQR) PG-SGA total score was also reported and paired scores comparisons were undertaken using Wilcoxon non-parametric tests to establish any change in median PG-SGA total scores between time points. The '*persistence'* of malnutrition occurred when a patient was classed as SGA B or C at baseline and also at 12 months, with movement

between SGA B and SGA C allowed. The *'development'* of malnutrition occurred when a patient was classed as SGA A at baseline but SGA B or C at 12 months. To establish if malnutrition persisted or developed at the 12 month point, the SGA categories were compared at baseline and 12 months in those patients followed up at the latter time point.

To determine if the presence/absence of any one GI symptom was associated with nutritional status, 2-sided Pearson chi-square tests were used. For each GI symptom measured at each time point, a cross-tabulation was performed to compare those with (i.e. mild, moderate or severe) and without (i.e. none) the symptom with respect to SGA category (SGA A and SGA B+C) and percentage unintentional weight loss in past three to six months (< 5% and ≥ 5%). Percentage unintentional weight loss was used in this association analysis, as it is commonly used in clinical practice as a surrogate marker of nutritional status.

To check the relationship between the overall symptom status (GSRS total scores) and malnutrition status (PG-SGA total scores), a Spearman's rank correlation was performed for each time point. Notably, although PG-SGA total scores function to triage patients rather than determine their nutritional status, the ability of the PG-SGA score to predict SGA category is excellent: it has a sensitivity of 98%, a specificity of 82%, a positive predictive value of 95% and a negative predictive value of 93% (Bauer et al. 2002). There is a significant difference in the median PG-SGA total scores for each of the SGA classifications ($p< 0.001$), with the severely malnourished patients having the highest scores (Bauer et al. 2002). As such, it was felt appropriate to use PG-SGA total score as a measure of nutritional status in the correlation analysis. Data were visualised using scatter plots and Dancey and Reidy's categorisations aided the determination of the strength of the correlation: where 1 is a perfect correlation; 0.7-0.9 is a strong correlation; 0.4-0.6 is a moderate correlation; 0.1-0.3 is a weak correlation; 0 is no correlation (Dancey & Reidy 2004).

Descriptive analysis was undertaken to report the FFQ and Dietplan© data using mean (SD) daily intake of energy, macronutrients, fibre, 24 micronutrients and 14 food groups at each time

point. For those with three FFQs, repeated measures analysis of variance (ANOVA) was used to compare the intakes at the three time points. Where $p < 0.05$, a Bonferroni post-hoc test was performed to determine which time points differed, if any. At the individual level, the daily intake of energy was compared with the appropriate estimated average requirement (EAR), while protein, fibre and 18 micronutrients were compared with the appropriate reference nutrient intake (RNI) (Scientific Advisory Committee on Nutrition 2012; Committee on Medical Aspects of Food Policy 1991). This allowed the determination of the percentage of individuals meeting their individualised requirements at each study visit. Those meeting and not meeting their requirements for energy and protein were then analysed according to median (range) GSRS total score and SGA category (SGA A and B+C) to determine any trends in the data.

The number of cases of SIBO diagnosed during the 12 month study period was calculated as a percentage of all patients tested for SIBO, with a 95% CI. If the GHMBT was persistently negative but was stopped prematurely, this was considered an incomplete test.

3.4.3.3 Violations and Deviations

An inclusion criterion for the study was that the patient was planned for radical treatment for OG cancer. However, if the patient completed the baseline study components and then decided not to proceed with their planned treatment approach, their baseline measurements were still used in the analysis. However, the investigator withdrew the patient before the next study visit. For any patient who (a) withdrew/was withdrawn (b) was missed or (c) was lost to follow-up after the baseline visit, their measurements up until that point were used in the analysis.

3.5 Results

3.5.1 Data Checking

There was a high accuracy of data entry into the study's database, with an error rate of < 1%, which was considered acceptable.

3.5.2 Screening

The study opened on 18th November 2011. A total of 334 patients were screened at the weekly OG specialist MDT meetings between this date and 17th May 2013. Of these patients, 196 were excluded for the reasons shown in the flow diagram (Figure 3-1). The remaining 138 patients were eligible to take part, of which 58 (42%) declined.

In the group that declined, there were 38 (65.5%) males and 20 (34.5%) females, whose median (range) ages were 65 (40-88) years and 73 (47-83) years respectively. The ECOG performance status grading was recorded for the patients who declined to participate, as well as those who consented (Oken et al. 1982). Of those who declined, 17 (29.3%) had a performance status of 0, 32 (55.2%) had a performance status of 1, 6 (10.3%) of 2, 1 (1.7%) of 3, no patient had a performance status of 4 and the performance status was not recorded for 2 (3.5%). The ethnicities of the patients who declined to participate were as follows: White, 51 (88%); Asian/British Asian, 3 (5.2%); Black/Black British, 2 (3.4%); Other, 2 (3.4%).

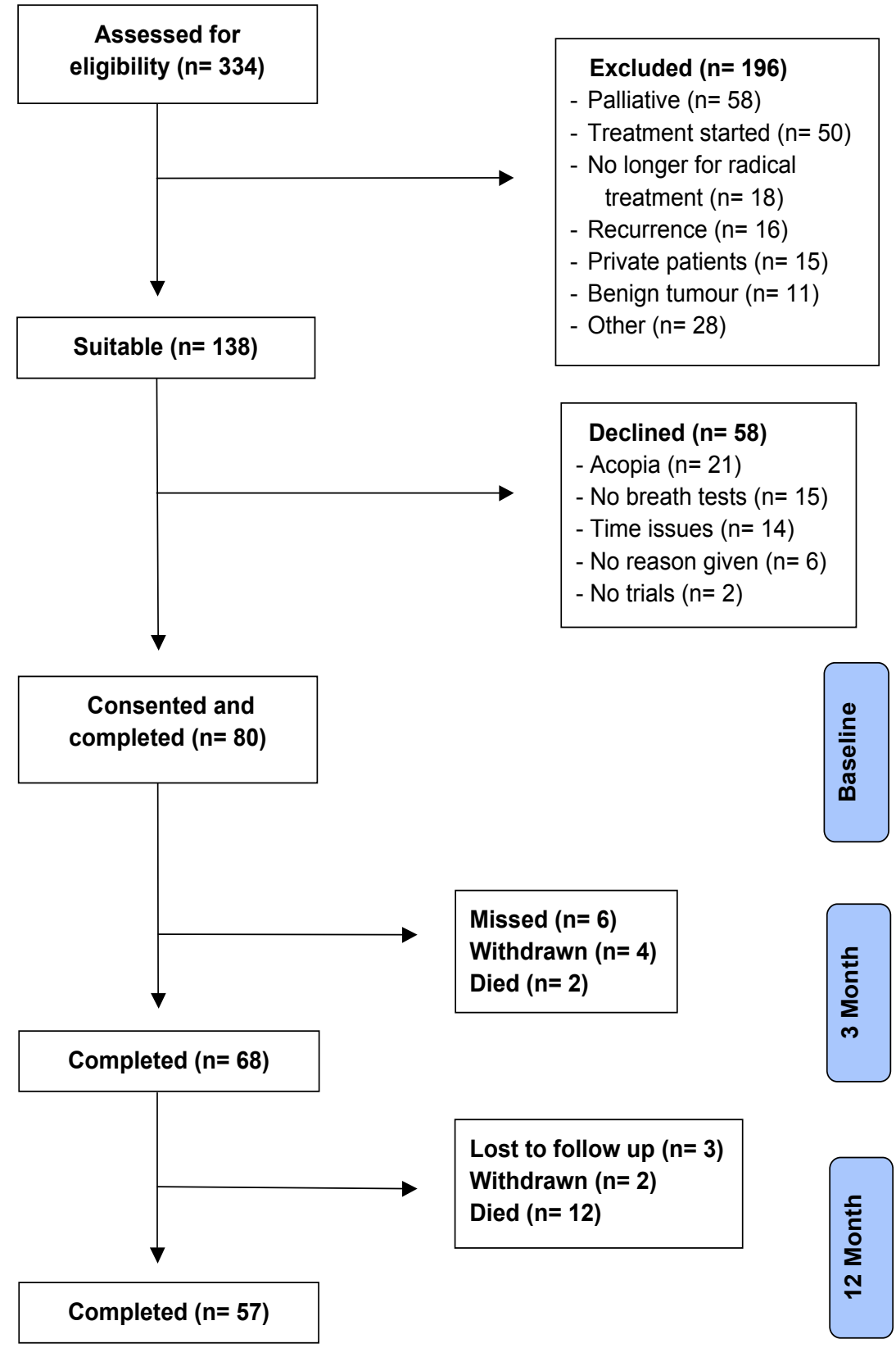


Figure 3-1 CCR 3703: screening and flow of patients through the study

3.5.3 Enrolment and Baseline Patient Characteristics

In total, the study dietitian recruited 80 patients into the study. The flow of these patients through the study is shown in Figure 3-1. There were 61 (76.2%) males and 19 (23.8%) females, whose baseline characteristics are described in Table 3-2. Of those who consented, 68 (85%) and 57 (71.3%) were followed up at 3- and 12 months respectively. There were 52 (65%) patients who were assessed at all three study visits.

Table 3-2 Baseline characteristics of the recruited cohort with oesophagogastric cancer

	n= 80
Age (years)	***median (range)***
Males	66 (47-89)
Females	61 (46-80)
Ethnicity	***n (%)***
White	70 (87.5)
Black/Black British	5 (6.3)
Asian/British Asian	4 (5)
Other	1 (1.2)
Previous gastrointestinal diagnoses	***n (%)***
Irritable bowel syndrome	5 (6.3)
Inflammatory bowel disease	1 (1.3)
Colorectal cancer (with colostomy)	1 (1.3)
Eastern Cooperative Oncology Group performance status	***n (%)***
0	35 (43.8)
1	39 (48.8)
2	5 (6.2)
3	1 (1.2)
4	0 (0)
Current diagnosis	***n (%)***
Adenocarcinoma of the upper and middle third of the oesophagus	23 (28.7)
Adenocarcinoma of the lower third of the oesophagus and Siewert type I tumour	14 (17.5)
Squamous cell carcinoma of the oesophagus	12 (15.0)
Siewert type II and III tumour	6 (7.5)
Adenocarcinoma of the stomach	15 (18.8)
Gastrointestinal stromal tumour of the stomach	5 (6.2)
Barrett's oesophagus	1 (1.3)
Other malignant/pre-malignant neoplasm	4 (5.0)
Histopathological tumour (T) staging	***n (%)***
0-1	6 (7.5)
2	14 (17.5)
3	48 (60.0)
4	5 (6.3)
Not applicable	7 (8.7)

3.5.4 Treatment Modalities

The intended treatment modalities at baseline (as per MDT decision) and the actual treatment received between (a) baseline and 3 months and (b) baseline and 12 months for those followed-up is described in Figure 3-2. Of those followed up at 3- and 12 months, 64 (94.1%) and 17 (29.8%) respectively were planned for further treatment after these time points.

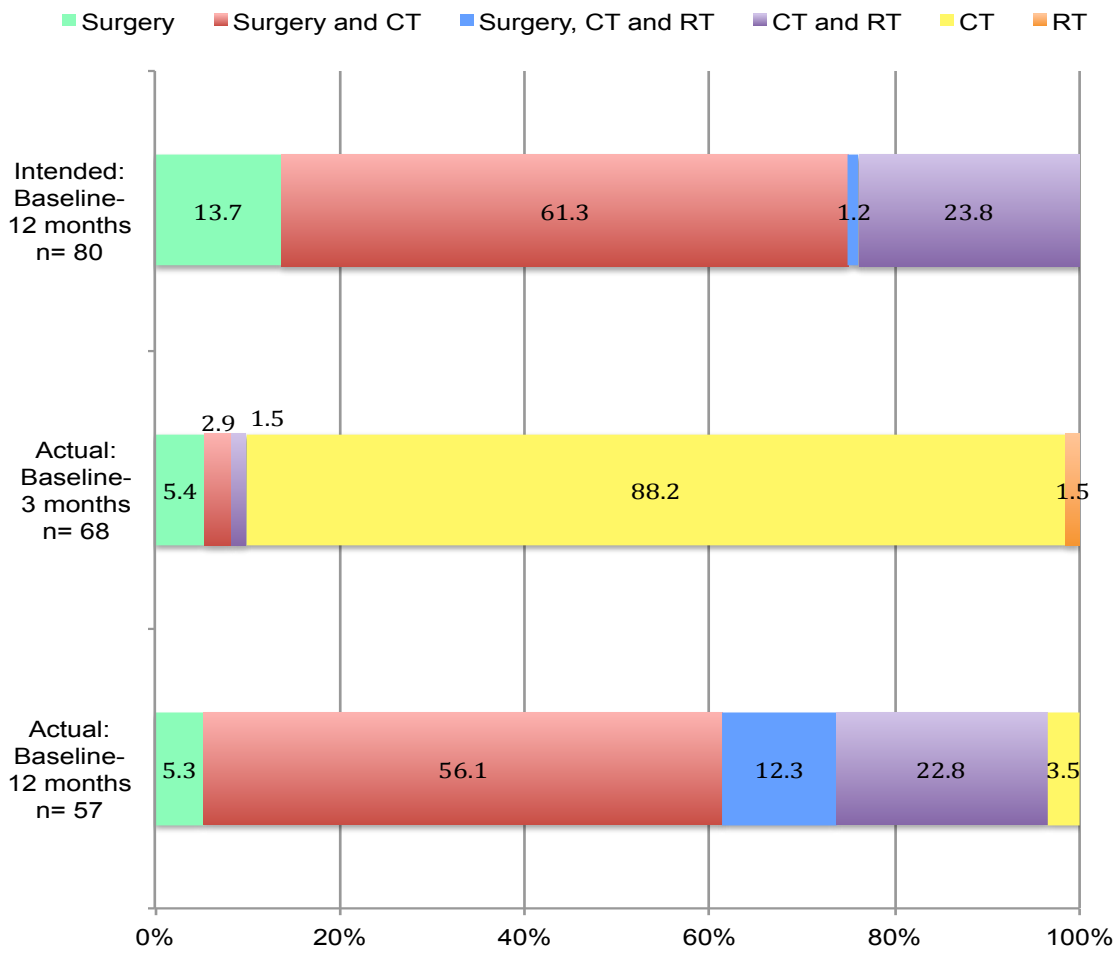


Figure 3-2 Treatment modalities intended at baseline, actual treatment received between baseline and 3 months and between baseline and 12 months for those followed-up, where CT is chemotherapy and RT is radiotherapy

At 12 months, of the 57 patients followed up, there were 42 (73.6%) who had undergone surgery- the surgery types and reconstruction methods are outlined in Table 3-3.

Table 3-3 Surgical procedures and reconstruction methods for the 42 patients who underwent a radical resection and were followed up at 12 months

Surgery type and reconstruction method	Number (%)
2 Phase Ivor Lewis Oesophagectomy with gastric conduit	2 (4.8)
2 Phase Ivor Lewis Oesophagogastrectomy with gastric conduit	20 (47.6)
3 Phase McKeown Oesophagectomy with gastric conduit	2 (4.8)
Total gastrectomy with Roux-en-Y anastomosis	8 (19)
Subtotal gastrectomy with Roux-en-Y anastomosis	5 (11.9)
Other	5 (11.9)

3.5.5 Gastrointestinal Symptoms

The proportions of patients reporting the 22 GSRS symptoms as none, mild, moderate and severe at the three study time points are displayed in Figure 3-3, Figure 3-4 and Figure 3-5. The most commonly reported symptom at each time point was flatulence: 78%, 65% and 70% reported it (as either mild, moderate or severe) at baseline, 3- and 12-months respectively. As the study progressed, the presence of dysphagia to solids and fluids reduced: at baseline 58% had dysphagia to solids and this fell to 37% by 12 months; at baseline 33% had dysphagia to fluids and this fell to 18% by 12 months. The opposite was true for diarrhoea and faecal incontinence: between baseline and 12 months there were 27% and 13% more patients experiencing them respectively.

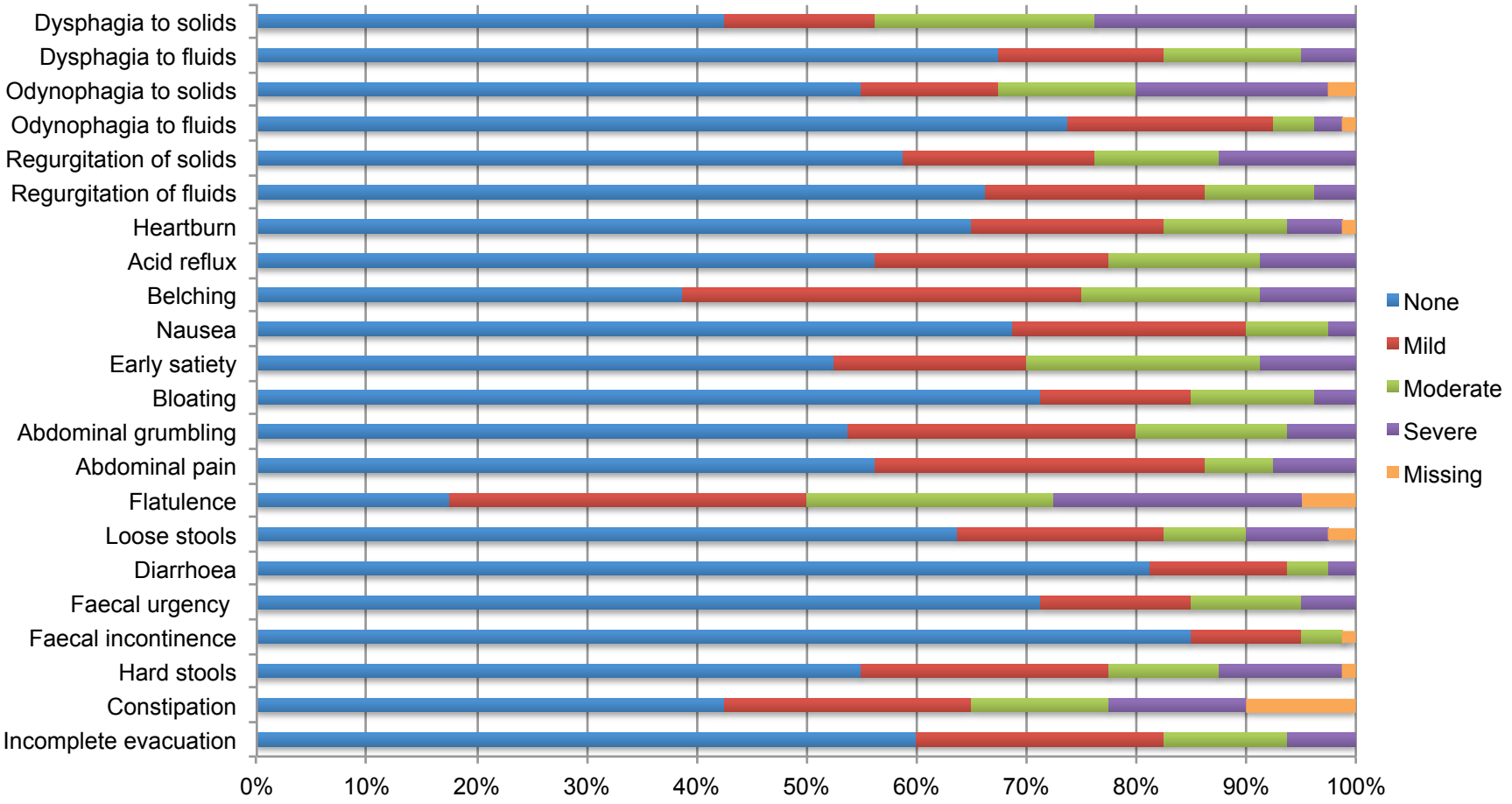


Figure 3-3 The proportion of none, mild, moderate, severe and missing gastrointestinal symptoms at baseline measured using the Gastrointestinal Symptom Rating Scale (n= 80)

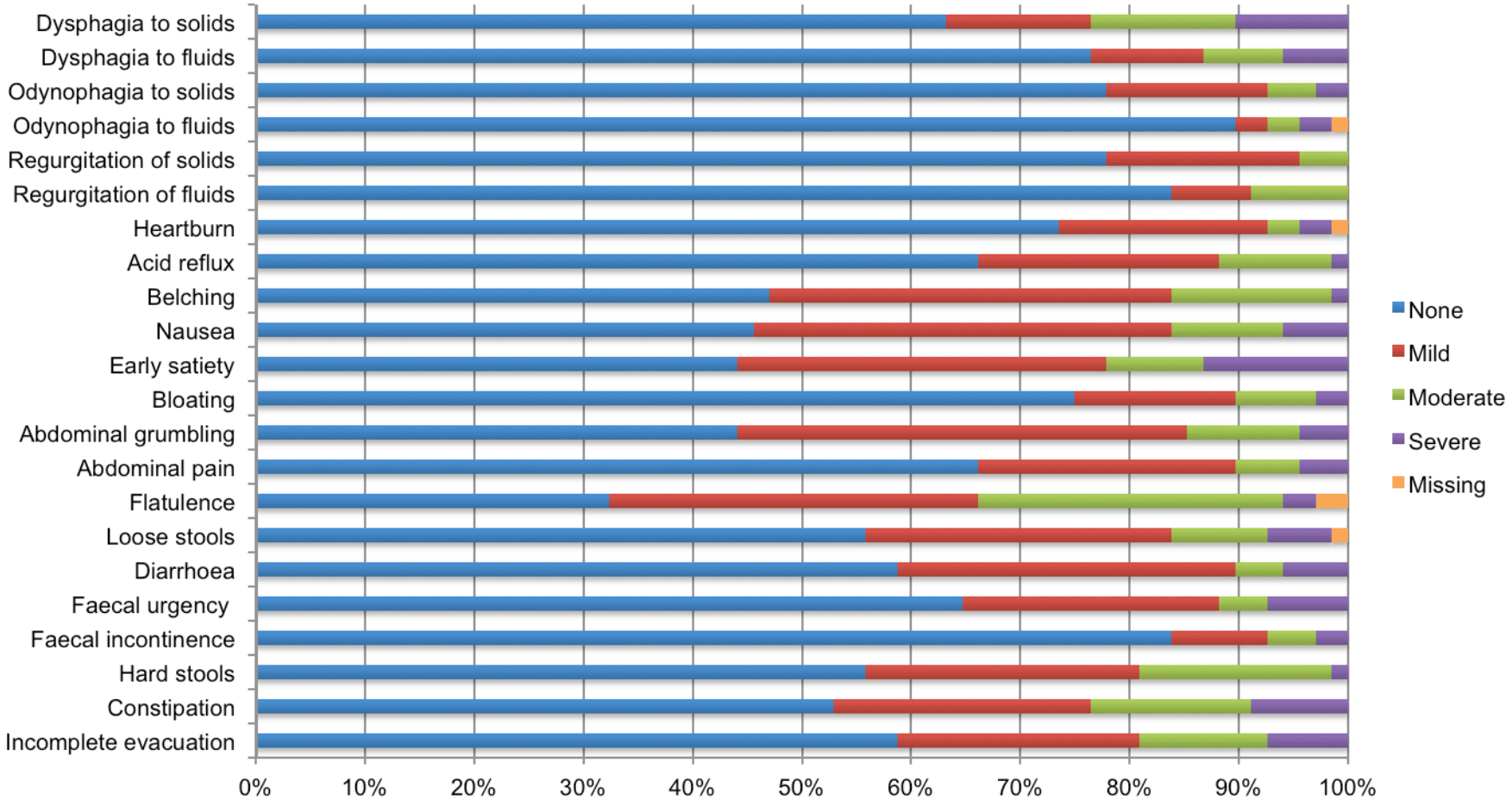


Figure 3-4 The proportion of none, mild, moderate, severe and missing gastrointestinal symptoms at 3 months measured using the Gastrointestinal Symptom Rating Scale (n= 68)

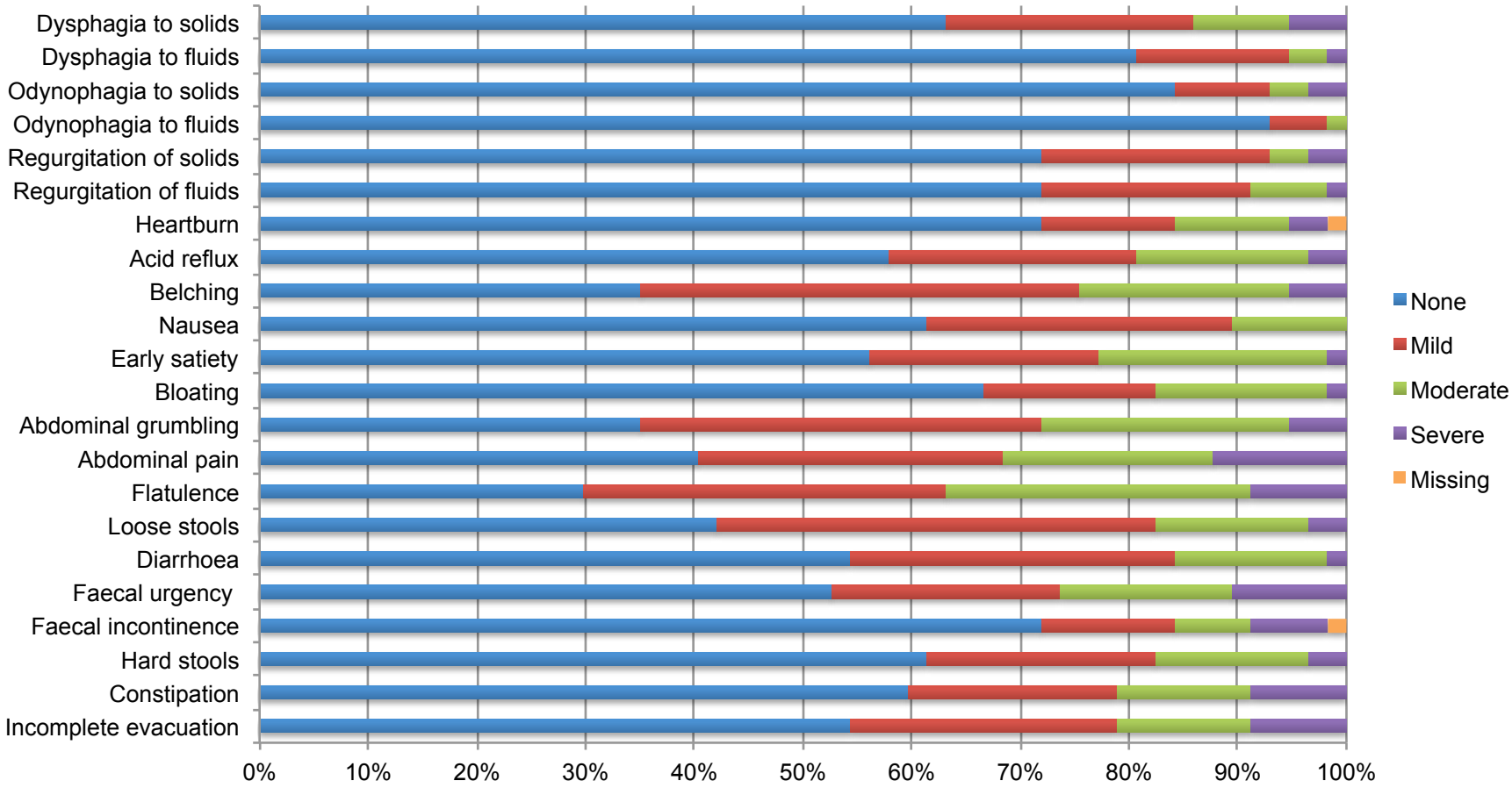


Figure 3-5 The proportion of none, mild, moderate, severe and missing gastrointestinal symptoms at 12 months measured using the Gastrointestinal Symptom Rating Scale (n= 57)

For the 80, 68 and 57 patients at baseline, 3- and 12 months, the median (range) number of GI symptoms of any severity was 8 (0-22), 8 (0-19) and 8 (0-20) respectively. The median (range) GSRS total scores were 12 (0-46), 9.5 (0-39) and 12 (0-46) at the three time points and the medians (IQRs) are depicted in Figure 3-6.

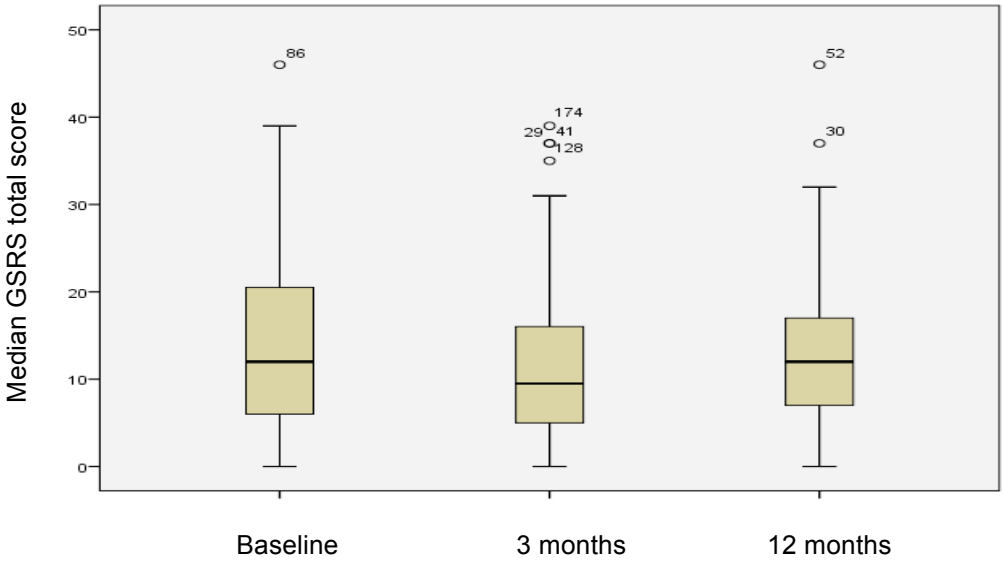


Figure 3-6 Median (IQR) Gastrointestinal Symptom Rating Scale total scores at baseline (n= 80), 3 months (n= 68) and 12 months (n= 57)

Paired score comparisons were performed for those with two or more GSRS questionnaires at any of the three time points, as shown in Table 3-4. There was a significant reduction in median GSRS total scores between baseline and 3 months.

Table 3-4 Paired score comparisons between study visits for Gastrointestinal Symptom Rating Scale total scores

n=	Baseline	3 months	12 months	p-value
68	12.5 (0-46)	9.5 (0-39)		0.028
57	12 (0-39)		12 (0-46)	0.993
52		9.5 (0-39)	12.5 (0-46)	0.240
Notes: Data expressed as median (range). Wilcoxon non-parametric tests were performed.				

There were 52 patients who completed the three GSRS questionnaires at the relevant study time points. Figure 3-7 displays the individual change in GI symptoms over the 12 months, with each of these patients represented by a line on the chart.

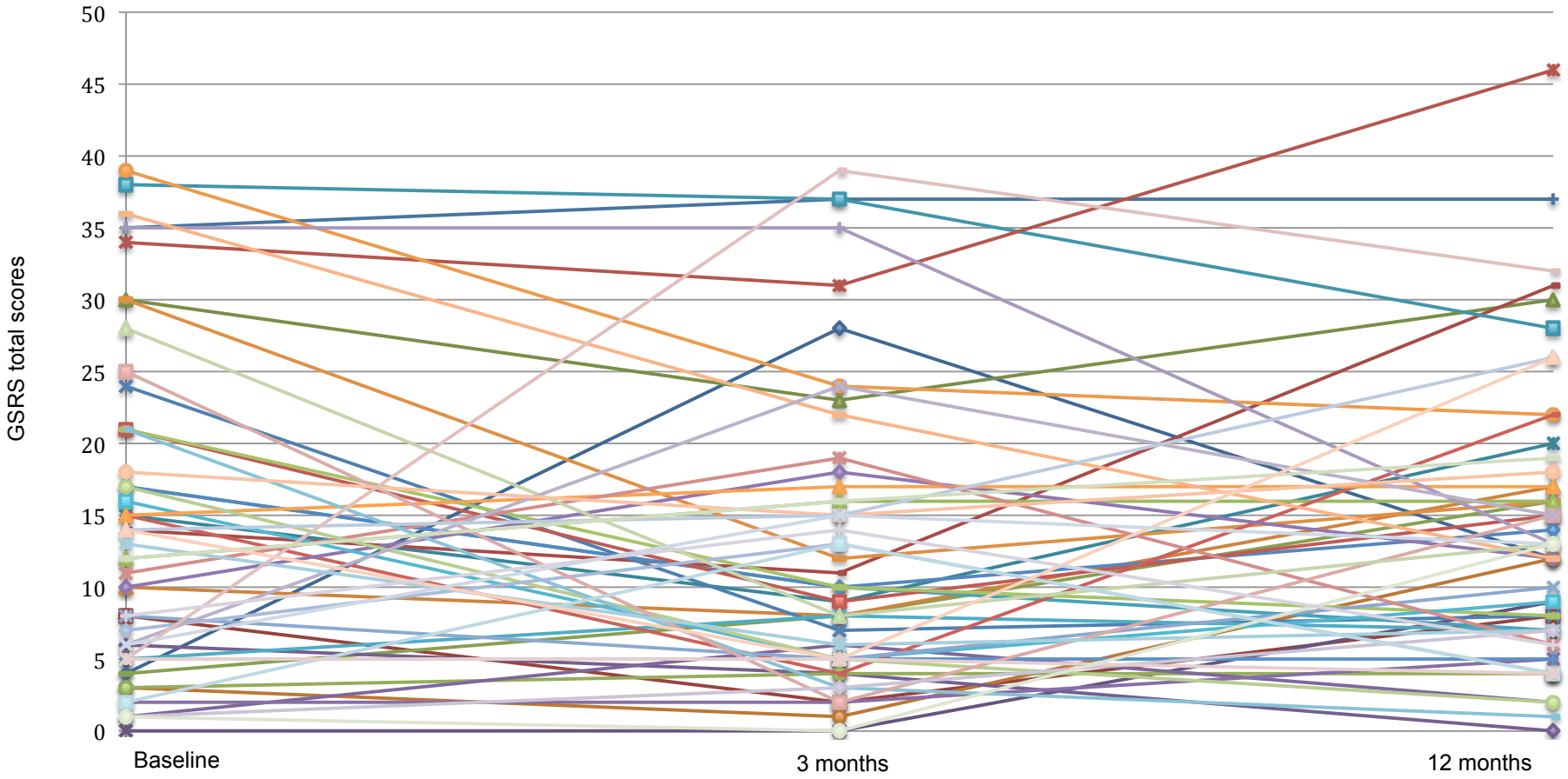


Figure 3-7 Gastrointestinal Symptom Rating Scale total scores for the 52 patients with complete symptom data: each line on the chart represents a patient

For the 52 patients with complete GI symptom data, the count (percentage) of those reporting moderate or severe individual GI symptoms is displayed in Table 3-5. Of note, at least 20% reported early satiety, constipation and incomplete evacuation of a moderate/severe degree at all study visits. Also, at least 20% experienced belching, flatulence and abdominal grumbling deemed moderate/severe at baseline and 12 months.

Table 3-5 The prevalence of moderate or severe symptoms in the 52 patients with complete symptom data

Symptom	Baseline	3 months	12 months
Dysphagia to solids	22 (42.3%)	11 (21.1%)	8 (15.4%)
Dysphagia to fluids	9 (17.3%)	7 (13.5%)	3 (5.8%)
Odynophagia to solids	16 (30.8%)	5 (9.6%)	4 (7.7%)
Odynophagia to fluids	4 (7.7%)	4 (7.7%)	1 (1.9%)
Regurgitation of solids	12 (23.1%)	3 (5.8%)	4 (7.7%)
Regurgitation of fluids	8 (15.4%)	5 (9.6%)	5 (9.6%)
Heartburn, n= 51	9 (17.6%)	3 (5.9%)	7 (13.7%)
Acid reflux	12 (23%)	6 (11.5%)	10 (19.2%)
Belching	13 (25%)	8 (15.4%)	14 (26.9%)
Nausea	6 (11.5%)	9 (17.3%)	5 (9.6%)
Early satiety	16 (30.8%)	11 (21.1%)	12 (23.1%)
Bloating	13 (25%)	7 (13.5%)	8 (15.4%)
Abdominal grumbling	12 (23.1%)	8 (15.4%)	14 (26.9%)
Abdominal pain	8 (15.4%)	7 (13.5%)	16 (30.8%)
Flatulence	18 (34.6%)	8 (15.4%)	20 (38.5%)
Loose stools	4 (7.7%)	8 (15.4%)	8 (15.4%)
Diarrhoea	2 (3.8%)	6 (11.5%)	7 (13.7%)
Faecal urgency	10 (19.2%)	7 (13.5%)	14 (26.9%)
Faecal incontinence	2 (3.8%)	4 (7.7%)	7 (13.5%)
Hard stools, n= 51	12 (23.5%)	10 (19.6%)	10 (19.6%)
Constipation	12 (23.1%)	12 (23.1%)	11 (21.1%)
Incomplete evacuation	11 (21.1%)	11 (21.1%)	11 (21.1%)
Key: Green= < 5% of patients reported the symptom as moderate or severe Orange= ≥ 5% - < 20% of patients reported the symptom as moderate or severe Red= ≥ 20% of patients reported the symptom as moderate or severe			

There were 57 patients with symptom data available at baseline and 12 months, of which 41 (71.9%) had at least one moderate or severe symptom at 12 months and the median (range) number of moderate/severe symptoms was 4 (0-17). To determine which symptoms persisted or developed between baseline and 12 months, the individual symptoms of these 57 patients were compared at both time points (Table 3-6). There were eight symptoms reported as moderate/severe at 12 months in ≥ 20% of patients (red shading). Conversely, there was just

one symptom where < 5% of patients reported it as moderate/severe at 12 months- odynophagia to fluids (green shading).

Table 3-6 The proportion of patients with persistence or new development of moderate-severe symptoms at 12 months as compared with baseline (n= 57)

	N (%) persistent moderate/severe symptom at 12 months	N (%) newly developed moderate/severe symptom at 12 months	N (%) persistent or newly developed moderate/severe symptom at 12 months
Dysphagia to solids	6 (10.5)	2 (3.5)	8 (14)
Dysphagia to fluids	1 (1.8)	2 (3.5)	3 (5.3)
Odynophagia to solids	4 (7)	0 (0)	4 (7)
Odynophagia to fluids	1 (1.8)	0 (0)	1 (1.8)
Regurgitation of solids	3 (5.3)	1 (1.8)	4 (7)
Regurgitation of fluids	2 (3.5)	3 (5.3)	5 (8.8)
Heartburn, n= 56	4 (7.1)	4 (7.1)	8 (14.2)
Acid reflux	5 (8.8)	6 (10.5)	11 (19.3)
Belching	6 (10.5)	8 (14)	14 (24.5)
Nausea	0 (0)	6 (10.5)	6 (10.5)
Early satiety	5 (8.8)	8 (14)	13 (22.8)
Bloating	3 (5.3)	7 (12.3)	10 (17.6)
Abdominal grumbling	4 (7)	12 (21.1)	16 (28.1)
Abdominal pain	4 (7)	14 (24.6)	18 (31.6)
Flatulence	13 (22.8)	8 (14)	21 (36.8)
Loose stools	2 (3.5)	8 (14)	10 (17.5)
Diarrhoea	2 (3.5)	7 (12.3)	9 (15.8)
Faecal urgency	6 (10.5)	9 (15.8)	15 (26.3)
Faecal incontinence, n= 56	1 (1.8)	7 (12.5)	8 (14.3)
Hard stools, n= 56	5 (8.9)	4 (7.1)	9 (16)
Constipation	6 (10.5)	6 (10.5)	12 (21)
Incomplete evacuation	6 (10.5)	6 (10.5)	12 (21)

Key:
Green= < 5% of patients reported the symptom as moderate/severe at 12 months
Orange= ≥ 5% - < 20% of patients reported the symptom as moderate/severe at 12 months
Red= ≥ 20% of patients reported the symptom as moderate/severe at 12 months

Bristol Stool Form Scale results are reported in Figure 3-8. There was a trend towards less hard stools (Type 1 or 2) and more loose stools (Type 6 or 7) between the baseline and 12 month study visits for both *'at best'* and *'at worst'* stool habit.

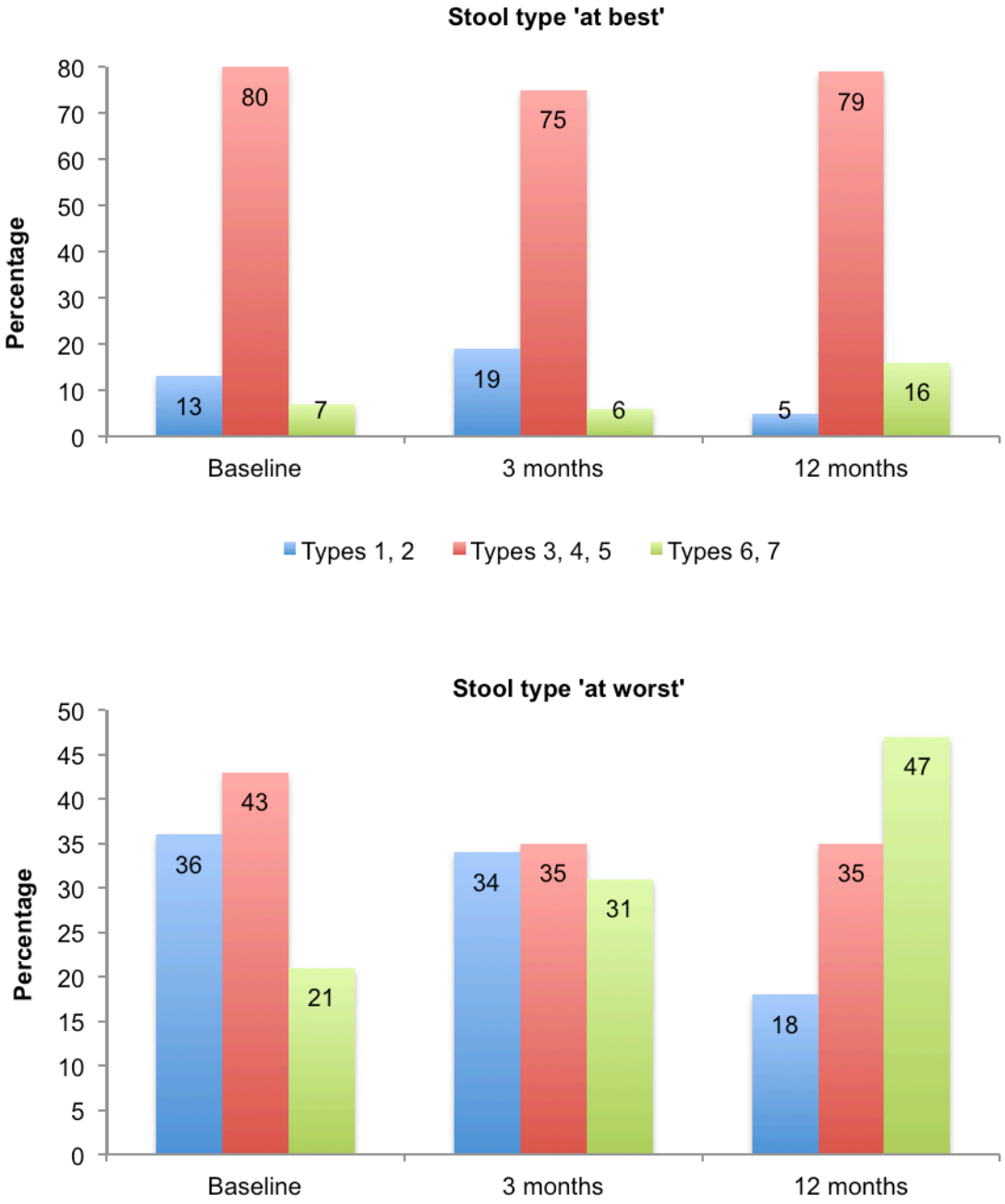


Figure 3-8 Bristol Stool Form Scale stool types reported when *'at best'* and *'at worst'* for baseline (n= 80), 3 months (n= 68) and 12 months (n= 57)

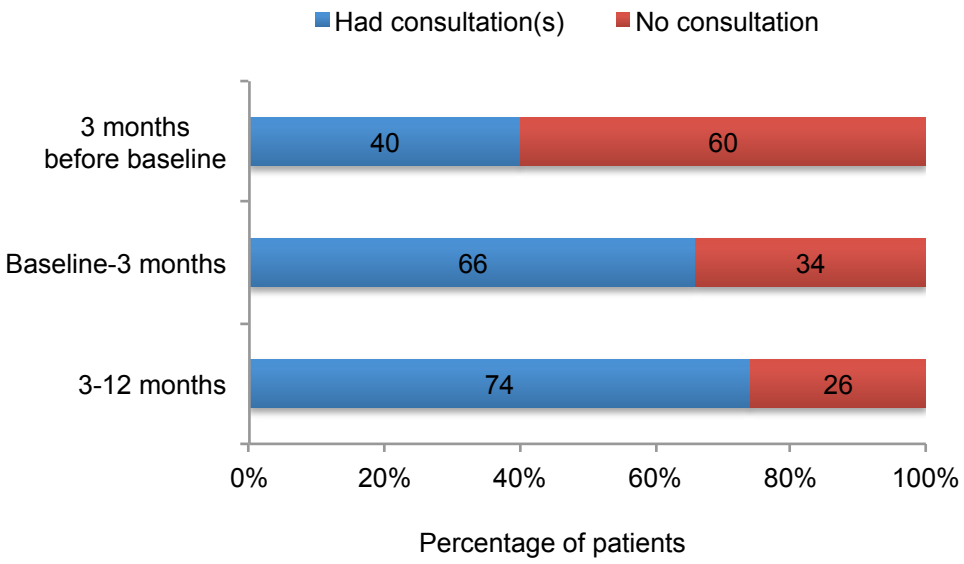
3.5.5.1 Hypothesis 1: Persistence or Development of Gastrointestinal Symptoms

Moderate-severe GI symptoms persisted or developed during the 12 month study period in this cohort of OG cancer patients. For the 57 who were followed up at 12 months, the comparison between baseline and 12 months for GSRS total scores indicates that there was no statistically

significant difference between the study visits, p= 0.993 (Table 3-4). The median (range) change between the two time points for GSRS total score was -1 (-27: 24). Of these 57 patients, 71.9% had at least one symptom that was moderate or severe at 12 months. The prevalence of persistent or newly developed individual symptoms ranged from 1.8% (odynophagia to fluids) to 36.8% (flatulence). Based on these results, Hypothesis 1 can be accepted.

3.5.6 Nutritional Status

The proportion of patients who had a least one clinical dietetic consultation in the 3 months before baseline and between study time points is reported in Figure 3-9. In total, 32 (40%) patients received an Eating Well When You Have Cancer booklet. The number (percentage) of patients who received it before baseline, between baseline and 3 months and between 3- and 12 months were: 14 (17.5), 16 (20) and 2 (2.5).



Number of Consultations

	n (%) that had consultation(s)	Mean (SD) number of consultations
3 months before baseline	32 (40)	1.6 (0.9)
Between baseline and 3 months	45 (66)	3.2 (4.3)
Between 3- and 12 months	42 (74)	7.9 (7.4)

Figure 3-9 The proportion of patients having at least one dietetic consultation in the 3 month period before baseline (n= 80), in the baseline-3 month period (n= 68) and in the 3-12 month period (n= 57): for those who had a consultation, the mean (SD) number of consultations is reported

The mean (SD) weights and BMIs for patients at baseline (n= 80), 3 months (n= 68) and 12 months (n= 57) were: 76.6 (17.2) kg and 26.7 (4.7) kg/m^2; 74.4 (14.8) kg and 25.9 (4.1) kg/m^2; 71.6 (16.7) kg and 25 (4.9) kg/m^2 respectively. Paired score comparisons were performed for those with data available at two (or more) time points, with significant reductions in weight and BMI for all three comparisons (Table 3-7).

Table 3-7 Paired scores comparisons between study visits for weight and body mass index

Mean (SD)	n=	Baseline	3 months	12 months	p-value
Weight, kg	68	76.3 (15.5)	74 (14.8)		0.003
BMI, kg/m^2		26.5 (4.4)	26 (4.1)		0.006
Weight, kg	57	77.6 (17.9)		71.6 (16.7)	<0.001
BMI, kg/m^2		27 (5)		25 (4.9)	<0.001
Weight, kg	52		74 (14.4)	70 (13.6)	<0.001
BMI, kg/m^2			26 (4.1)	24.5 (4.1)	<0.001
Note: Paired t-tests were performed					

The scores from all components of PG-SGA, PG-SGA total scores and SGA categories are reported in Table 3-8. With reference to Worksheet 4 (nutrition-related physical examination and anthropometric assessment), at least 30% of patients obtained a score at all time points, meaning that the depletion of fat and muscle stores (and/or the presence of ascites/oedema) contributed to malnutrition in these patients. Conversely, there was just one patient (at 3 months) who was given a metabolic demand score during the study (Worksheet 3). Of those assessed at baseline, 3- and 12 months, 61.2%, 61.8% and 59.6% were moderately/severely malnourished. The median (IQR) PG-SGA total scores are depicted in Figure 3-10.

Table 3-8 Patient Generated Subjective Global Assessment (PG-SGA) total scores and Subjective Global Assessment (SGA) categories at baseline, 3 months and 12 months

	Baseline n= 80	3 months n= 68	12 months n= 57
PG-SGA total score components		**n (%)**	
Box 1: *Weight*			
0 (= not changed/increased)	34 (42.5)	39 (57.3)	42 (73.7)
1 (= lost in past 2 weeks)	15 (18.7)	7 (10.3)	5 (8.8)
2	10 (12.5)	6 (8.8)	3 (5.3)
3	12 (15)	8 (11.8)	4 (7)
4	6 (7.5)	7 (10.3)	3 (5.2)
5	3 (3.8)	1 (1.5)	0 (0)
The higher the % lost in 1- or 6 months, the higher the score. Add 1 if some lost in past 2 weeks.			
Box 2: *Food intake*			
0 (= same/more than usual)	31 (38.8)	35 (51.5)	27 (47.4)
1 (= less food than usual)	29 (36.2)	20 (29.4)	24 (42.1)
2 (= little solid food)	11 (13.7)	11 (16.1)	6 (10.5)
3 (= supplements only)	9 (11.3)	1 (1.5)	0 (0)
4 (= very little of anything)	0 (0)	1 (1.5)	0 (0)
Box 3: *Symptoms*			
0-3 (= none/few symptoms)	43 (53.7)	45 (66.2)	35 (61.4)
4-6 (= several symptoms)	11 (13.8)	13 (19.1)	10 (17.5)
7+ (= many symptoms)	26 (32.5)	10 (14.7)	12 (21.1)
Box 4: *Activities and function*			
0 (= no limitations)	53 (66.2)	22 (32.4)	31 (54.4)
1 (= not normal self)	18 (22.5)	26 (38.2)	15 (26.4)
2 (= not up to most things)	7 (8.8)	11 (16.2)	7 (12.2)
3 (= able for little activity)	2 (2.5)	9 (13.2)	4 (7)
Sum of Boxes 1-4			
0-6	37 (46.3)	44 (64.7)	38 (66.7)
7-12	28 (35)	14 (20.6)	14 (24.5)
13-18	10 (12.4)	9 (13.2)	5 (8.8)
19-24	5 (6.3)	1 (1.5)	0 (0)
Median (range)	7 (0-24)	4 (0-23)	4 (0-16)
Worksheet 2: *Relevant diagnoses*			
0 (= no diagnoses)	2 (2.5)	0 (0)	3 (5.3)
1 (= one diagnosis)	32 (40)	26 (38.2)	25 (43.9)
2 (= two diagnoses)	46 (57.5)	41 (60.3)	28 (49.1)
3 (= three diagnoses)	0 (0)	1 (1.5)	1 (1.7)
Worksheet 3: *Metabolic demand*			
0 (= no demand)	80 (100)	67 (98.5)	57 (100)
1 (= mild demand)	0 (0)	1 (1.5)	0 (0)
Worksheet 4: *Physical examination*			
0 (= no deficit)	53 (66.2)	47 (69.1)	40 (70.2)
1 (= mild deficit)	19 (23.8)	15 (22.1)	11 (19.3)
2 (= moderate deficit)	8 (10)	6 (8.8)	5 (8.8)
3 (= severe deficit)	0 (0)	0(0)	1 (1.7)
PG-SGA total score, median (range)	9 (0-28)	6 (2-26)	7 (0-19)
SGA category		**n (%)**	
A: Well-nourished	31 (38.8)	26 (38.2)	23 (40.4)
B: Moderately/suspected malnourished	47 (58.7)	40 (58.8)	32 (56.1)
C: Severely malnourished	2 (2.5)	2 (3)	2 (3.5)

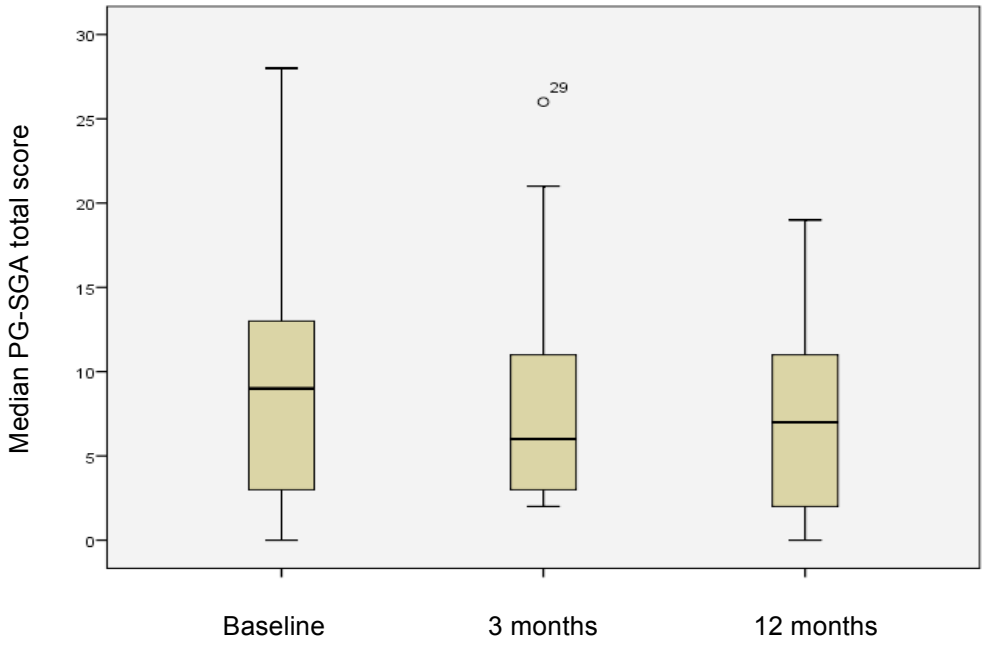


Figure 3-10 Median (IQR) Patient Generated Subjective Global Assessment total scores at baseline (n= 80), 3 months (n= 68) and 12 months (n= 57)

Paired PG-SGA total scores comparisons were performed for patients with two or more complete assessments, with no significant differences noted between any of the study visits (Table 3-9).

Table 3-9 Paired scores comparisons between study visits for Patient Generated Subjective Global Assessment total scores

Median (range)	n=	Baseline	3 months	12 months	p-value
PG-SGA total score	68	9 (0-28)	6 (2-26)		0.095
	57	8 (0-23)		7 (0-19)	0.368
	52		5 (2-64)	7 (1-19)	0.730
Note: Wilcoxon non-parametric tests were performed					

To determine whether individuals moved or stayed within an SGA category between time points, the SGA category trends are shown in Table 3-10. As can be seen, 16 (23.5%) experienced an improvement in their SGA category from baseline to 3 months (shaded green), whereas 16 (23.5%) worsened their SGA category (shaded red). From baseline to 12 months, 14 (24.6%) experienced an improvement in their category (shaded green), while 16 (28%) worsened their category (shaded red).

Table 3-10 Cross-tabulation of Subjective Global Assessment categories at (a) baseline and 3 months, (b) baseline and 12 months and (c) 3 months and 12 months

Baseline - 3 months		Baseline			
		SGA A	SGA B	SGA C	Total
3 months	SGA A	11	14	1	26
	SGA B	14	25	1	40
	SGA C	0	2	0	2
	Missing	6	6	0	12
	Total	31	47	2	80

Baseline - 12 months		Baseline			
		SGA A	SGA B	SGA C	Total
12 months	SGA A	10	13	0	23
	SGA B	14	17	1	32
	SGA C	1	1	0	2
	Missing	6	16	1	23
	Total	31	47	2	80

3 months - 12 months		3 months				
		SGA A	SGA B	SGA C	Missing	Total
12 months	SGA A	8	12	0	3	23
	SGA B	14	14	2	2	32
	SGA C	0	2	0	0	2
	Missing	4	12	0	7	23
	Total	26	40	2	12	80

Notes: SGA A= well-nourished; SGA B= moderately/suspected malnourished; SGA C= severely malnourished. Data expressed as number of patients.
Key: Green= improved nutritional status; Red= worsened nutritional status

3.5.6.1 Hypothesis 2: Persistence or Development of Malnutrition

Malnutrition persisted or developed during the 12 month study period in this cohort of OG cancer patients. For the 57 patients with baseline and 12 month nutritional status data, the paired score comparison for PG-SGA total scores indicates that there was no statistically significant difference between the study visits, p= 0.368 (Table 3-9). The median (range) change between the time points for this score was 1 (-13: 16). Thirteen (22.8%) patients moved from moderately/suspected malnourished at baseline to well-nourished at 12 months, 19 (33.3%) were moderately/severely malnourished at both time points (malnutrition '*persisted'*) and 15 (26.4%) were well-nourished to start but became moderately/ severely malnourished by 12 months (malnutrition *'developed'*) (Table 3-10). As such, malnutrition either persisted or developed during the course of the study in the majority of this group: 34 of 57 patients (59.7%). Therefore, Hypothesis 2 can be accepted.

3.5.7 Association Between Gastrointestinal Symptoms and Nutritional Status

As has been reported, moderate-severe GI symptoms (Section 3.5.5) and malnutrition (Section 3.5.6) persisted or developed in the majority (71.9% and 59.6% respectively) of patients with OG cancer at 12 months. The association between each of the 22 GI symptoms and (a) SGA category and (b) percentage unintentional weight loss in the previous three-six months at each time point are reported in Table 3-11, Table 3-12 and Table 3-13.

Fewer symptoms were found to be associated with SGA and percentage weight loss at 3 months than were at baseline and fewer again at 12 months compared with 3 months: for SGA there were 11, 3 and 0 symptoms at baseline, 3- and 12 months respectively; for percentage weight loss, there were 7, 3 and 2 symptoms at baseline, 3- and 12 months respectively. Of note, dysphagia to solids, dysphagia to fluids, hard stools and constipation were found to be associated with both SGA category and percentage weight loss at baseline. Early satiety was the only symptom found to be associated with both SGA category and percentage weight loss at 3 months and there were no parallel associations at 12 months.

Table 3-11 Association between the presence of gastrointestinal symptoms (mild, moderate or severe) and Subjective Global Assessment (SGA) category and 3-6 month unintentional weight loss at baseline (n= 80)

Baseline	SGA, n (%)			Weight loss in previous 3-6 months, n (%)		
	SGA A	SGA B+C	p-value	< 5%	≥ 5%	p-value
Dysphagia to solids	11 (23.4)	36 (76.6)	0.001 **	23 (48.9)	24 (51.1)	0.006 **
Dysphagia to fluids	5 (19.2)	21 (80.8)	0.011 *	11 (42.3)	15 (57.7)	0.015 *
Odynophagia to solids	9 (26.5)	25 (73.5)	0.030 *	18 (52.9)	16 (47.1)	0.128
Odynophagia to fluids, n= 79	3 (15)	17 (85)	0.009 **	9 (45)	11 (55)	0.081
Regurgitation of solids, n= 78	9 (27.3)	24 (72.7)	0.062	14 (42.4)	19 (57.6)	0.004 **
Regurgitation of fluids	7 (25.9)	20 (74.1)	0.074	12 (44.4)	15 (55.6)	0.025 *
Heartburn, n= 79	12 (42.9)	16 (57.1)	0.401	17 (60.7)	11 (39.3)	0.523
Acid reflux	12 (34.3)	23 (65.7)	0.312	17 (48.6)	18 (51.4)	0.034 *
Belching	15 (30)	35 (70)	0.033 *	30 (60)	20 (40)	0.478
Nausea	3 (12)	22 (88)	0.001 **	14 (56)	11 (44)	0.342
Early satiety	7 (18.4)	31 (81.6)	0.000 ***	20 (52.6)	18 (47.4)	0.101
Bloating	8 (34.8)	15 (65.2)	0.420	14 (60.9)	9 (39.1%)	0.580
Abdominal grumbling	10 (27)	27 (73)	0.038 *	21 (56.8)	16 (43.2)	0.296
Abdominal pain	13 (37.1)	22 (62.9)	0.489	21 (60)	14 (40)	0.511
Flatulence, n= 76	16 (33.3)	32 (66.7)	0.163	29 (60.4)	19 (39.6)	0.520
Loose stools, n= 78	10 (43.5)	13 (56.5)	0.380	15 (65.2)	8 (34.8)	0.420
Diarrhoea	3 (20)	12 (80)	0.084	8 (53.3)	7 (46.7)	0.339
Faecal urgency	10 (43.5)	13 (56.5)	0.380	17 (73.9)	6 (26.1)	0.110
Faecal incontinence, n= 79	3 (27.3)	8 (72.7)	0.299	8 (72.7)	3 (27.3)	0.332
Hard stools, n= 79	8 (22.9)	27 (77.1)	0.007 **	16 (45.7)	19 (54.3)	0.013 *
Constipation, n= 72	6 (16.7)	30 (83.3)	0.000 ***	17 (47.2)	19 (52.8)	0.018 *
Incomplete evacuation	8 (25)	24 (75)	0.033 *	17 (53.1)	15 (46.9)	0.163

Notes: Pearson chi-square tests were undertaken to determine the association between individuals with/without a symptom and SGA category A or category B+C and weight loss < 5% or ≥ 5%. Fisher's Exact tests were performed where expected cell count was less than 5.
Data presented are for the association of the presence of a symptom and SGA B+C and weight loss ≥ 5%.
Key: * p< 0.05; ** p< 0.01; *** p< 0.001

Table 3-12 Association between the presence of gastrointestinal symptoms (mild, moderate or severe) and Subjective Global Assessment (SGA) category and 3-6 month unintentional weight loss at 3 months (n= 68)

3 months	SGA, n (%)			Weight loss in previous 3-6 months, n (%)		
	SGA A	SGA B+C	p-value	< 5%	≥ 5%	p-value
Dysphagia to solids	6 (24)	19 (76)	0.055	13 (52)	12 (48)	0.479
Dysphagia to fluids	4 (25)	12 (75)	0.171	6 (37.5)	10 (62.5)	0.103
Odynophagia to solids	4 (26.7)	11 (73.3)	0.231	8 (53.3)	7 (46.7)	0.577
Odynophagia to fluids, n= 67	1 (16.7)	5 (83.3)	0.240	2 (33.3)	4 (66.7)	0.242
Regurgitation of solids	3 (20)	12 (80)	0.087	7 (46.7)	8 (53.3)	0.348
Regurgitation of fluids	2 (18.2)	9 (81.8)	0.122	5 (45.5)	6 (54.5)	0.372
Heartburn, n= 67	6 (35.3)	11 (64.7)	0.482	9 (52.9)	8 (47.1)	0.523
Acid reflux	7 (30.4)	16 (69.6)	0.249	10 (43.5)	13 (56.5)	0.150
Belching	17 (47.2)	19 (52.8)	0.085	23 (63.9)	13 (36.1)	0.078
Nausea	14 (37.8)	23 (62.2)	0.569	21 (56.8)	16 (43.2)	0.429
Early satiety	8 (21.1)	30 (78.9)	0.001 **	14 (36.8)	24 (63.2)	0.001 **
Bloating	5 (29.4)	12 (70.6)	0.285	5 (29.4)	12 (70.6)	0.017 *
Abdominal grumbling	16 (42.1)	22 (57.9)	0.314	22 (57.9)	16 (42.1)	0.343
Abdominal pain	7 (30.4)	16 (69.6)	0.249	7 (30.4)	16 (69.6)	0.005 *
Flatulence	17 (38.6)	27 (61.4)	0.569	25 (56.8)	19 (43.2)	0.387
Loose stools, n= 67	12 (41.4)	17 (58.6)	0.449	15 (51.7)	14 (48.3)	0.483
Diarrhoea	9 (32.1)	19 (67.9)	0.271	16 (57.1)	12 (42.9)	0.448
Faecal urgency	10 (41.7)	14 (58.3)	0.431	15 (62.5)	9 (37.5)	0.232
Faecal incontinence	5 (45.5)	6 (54.5)	0.414	6 (54.5)	5 (0.412)	0.628
Hard stools	10 (33.3)	20 (66.7)	0.314	19 (63.3)	11 (36.7)	0.143
Constipation	8 (25)	24 (75)	0.030 *	17 (53.1)	15 (46.9)	0.517
Incomplete evacuation	6 (21.4)	22 (78.6)	0.015 *	12 (42.9)	16 (57.1)	0.088

Notes: Pearson chi-square tests were undertaken to determine the association between individuals with/without a symptom and SGA category A or category B+C and weight loss < 5% or ≥ 5%. Fisher's Exact tests were performed where expected cell count was less than 5.
Data presented are for the association of the presence of a symptom and SGA B+C and weight loss ≥ 5%.
Key: * p< 0.05; ** p< 0.01

Table 3-13 Association between the presence of gastrointestinal symptoms (mild, moderate or severe) and Subjective Global Assessment (SGA) category and 3-6 month unintentional weight loss at 12 months (n= 57)

12 months	SGA, n (%)			Weight loss in previous 3-6 months, n (%)		
	SGA A	SGA B+C	p-value	< 5%	≥ 5%	p-value
Dysphagia to solids	7 (33.3)	14 (66.7)	0.294	13 (61.9)	8 (38.1)	0.383
Dysphagia to fluids	3 (27.3)	8 (72.7)	0.264	7 (63.6)	4 (36.4)	0.537
Odynophagia to solids	2 (22.2)	7 (77.8)	0.204	3 (33.3)	6 (66.7)	0.030 *
Odynophagia to fluids	0 (0)	4 (100)	0.117	1 (25)	3 (75)	0.103
Regurgitation of solids	6 (37.5)	10 (62.5)	0.514	10 (62.5)	6 (37.5)	0.452
Regurgitation of fluids	6 (37.5)	10 (62.5)	0.514	10 (62.5)	6 (37.5)	0.452
Heartburn, n= 56	5 (33.3)	10 (66.7)	0.346	10 (66.7)	5 (33.3)	0.575
Acid reflux	10 (41.7)	14 (58.3)	0.539	15 (62.5)	9 (37.5)	0.386
Belching	13 (35.1)	24 (64.9)	0.209	22 (59.5)	15 (40.5)	0.100
Nausea	8 (36.4)	14 (63.6)	0.419	14 (63.6)	8 (36.4)	0.459
Early satiety	7 (28)	18 (72)	0.079	14 (56)	11 (44)	0.110
Bloating	9 (47.4)	10 (52.6)	0.315	15 (78.9)	4 (21.1)	0.137
Abdominal grumbling	14 (37.8)	23 (62.2)	0.402	24 (64.9)	13 (35.1)	0.465
Abdominal pain	12 (35.3)	22 (64.7)	0.251	22 (64.7)	12 (35.3)	0.465
Flatulence	14 (35)	26 (65)	0.166	26 (65)	14 (35)	0.465
Loose stools	10 (30.3)	23 (69.7)	0.062	19 (57.6)	14 (42.4)	0.076
Diarrhoea	8 (30.8)	18 (69.2)	0.140	16 (61.5)	10 (38.5)	0.319
Faecal urgency	8 (29.6)	19 (70.4)	0.097	17 (63)	10 (37)	0.389
Faecal incontinence n= 56	5 (33.3)	10 (66.7)	0.669	8 (53.3)	7 (46.7)	0.412
Hard stools	7 (31.8)	15 (68.2)	0.223	11 (50)	11(50)	0.034 *
Constipation	10 (43.5)	13 (56.5)	0.451	15 (65.2)	8 (34.8)	0.535
Incomplete evacuation	10 (38.5)	16 (61.5)	0.503	16 (61.5)	10 (38.5)	0.319

Notes: Pearson chi-square tests were undertaken to determine the association between individuals with/without a symptom and SGA category A or category B+C and weight loss < 5% or ≥ 5%. Fisher's Exact tests were performed where expected cell count was less than 5.
Data presented are for the association of the presence of a symptom and SGA B+C and weight loss ≥ 5%.
Key: * p< 0.05

To determine the correlation between combined burden of GI symptoms (as opposed to individual symptoms) and nutritional status, the GSRS total scores and PG-SGA total scores were compared. There was a correlation coefficient of +0.55 at baseline (n= 80, p< 0.001). At 3 months, where data for 68 patients were available, the correlation coefficient reduced to +0.51 (p< 0.001). At 12 months, where data for 57 patients were available, the correlation coefficient reduced further to +0.42 (p= 0.001). At each time point, the positive correlation between the variables is considered *'moderate'* as per Dancey and Reidy's correlation categorisations (Figure 3-11) (Dancey & Reidy 2004).

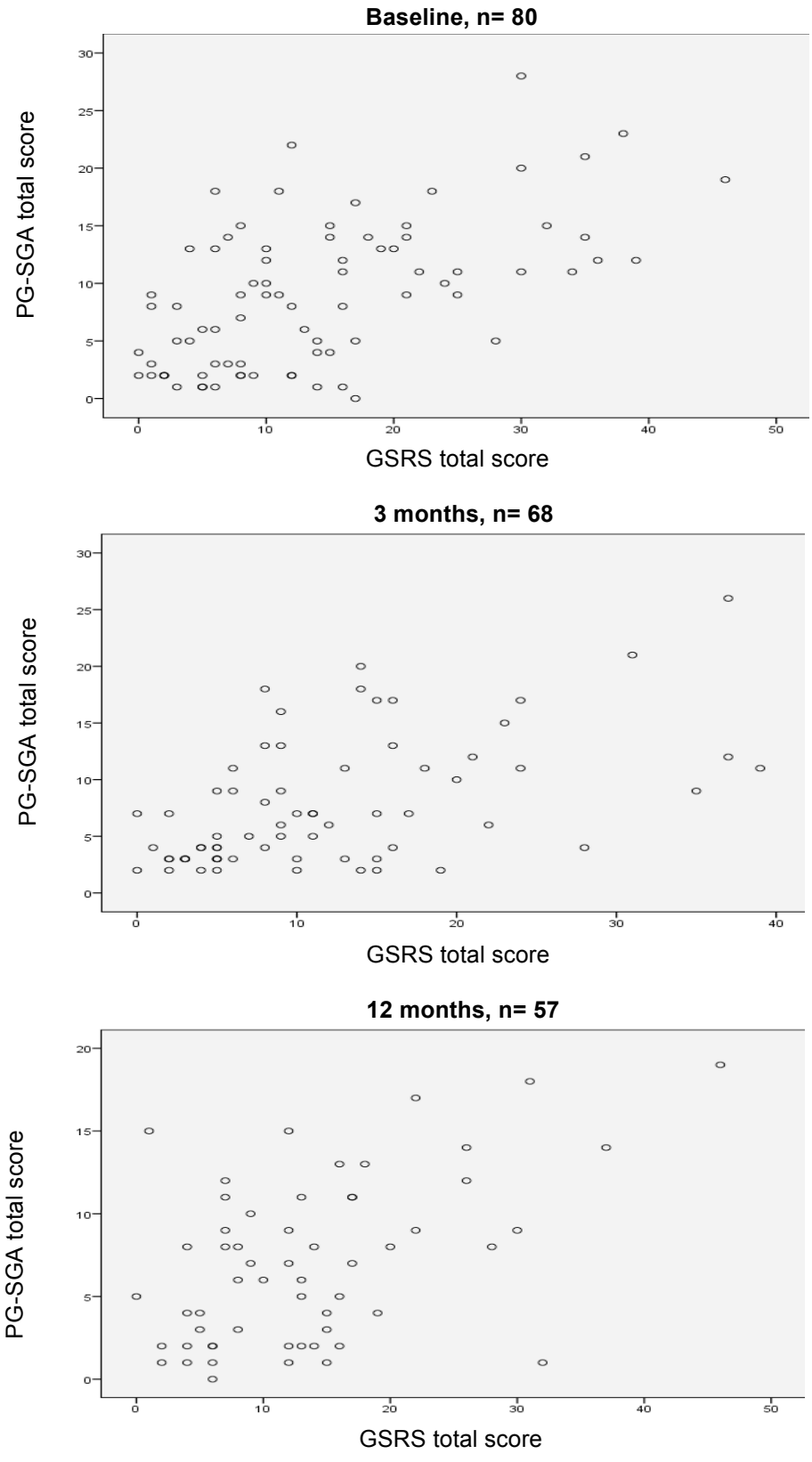


Figure 3-11 Spearman's rank correlation between Gastrointestinal Symptom Rating Scale total score and Patient Generated Subjective Global Assessment total score at: baseline (r= +0.55, p< 0.001); 3 months (r= +0.51, p< 0.001); and 12 months (r= +0.42, p= 0.001)

3.5.7.1 Hypothesis 3: Gastrointestinal Symptoms and Nutritional Status Association

A moderate positive correlation between GSRS total scores and PG-SGA total scores has been identified at baseline, during the acute phase of management (3 months) and chronically (12 months) in OG cancer patients. In addition, at each time point, association between some individual symptoms and both SGA category and percentage weight loss also occurs. Therefore, Hypothesis 3 can be accepted.

3.5.8 Dietary Assessment

For the 80, 68 and 57 patients assessed at baseline, 3- and 12 months, the sources of nutrition used during the month before each study visit is shown Figure 3-12. Of the 79, 62 and 53 managing (at least) some oral intake at baseline, 3- and 12 months, 18 (22.8%), 13 (21%) and 5 (9.4%) were consuming foods with a modified texture, either mostly or exclusively during the previous month. Of note, there were patients with an oesophageal stent in place at each time point: 3 (3.8%) at baseline, 5 (7.4%) at 3 months and 3 (5.3%) at 12 months.

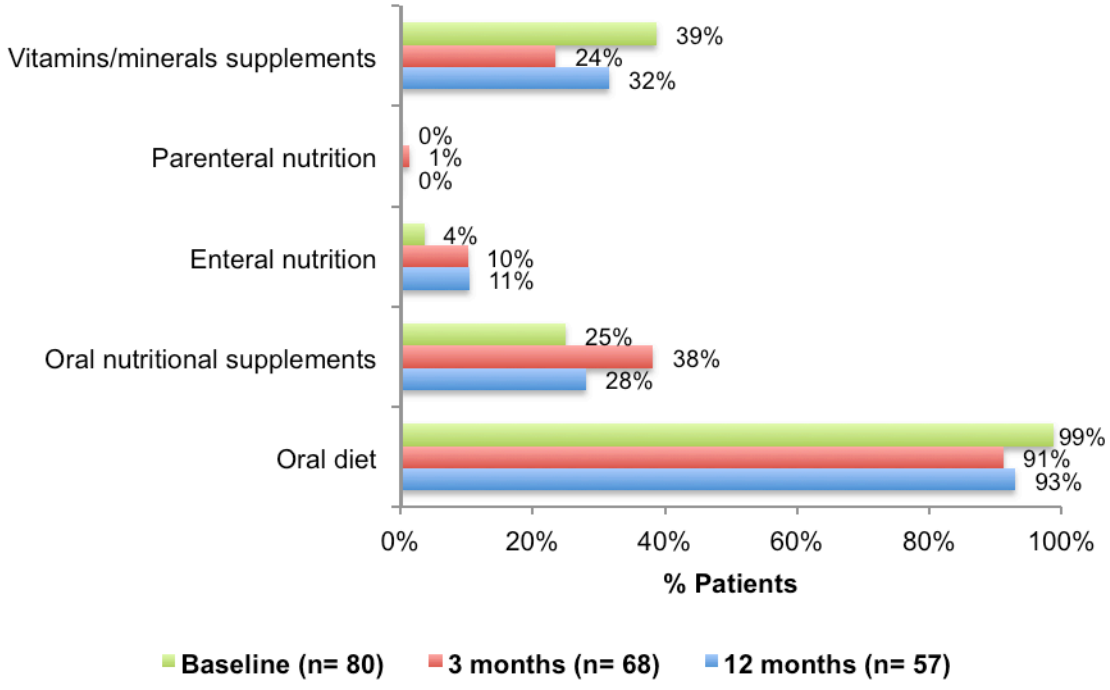


Figure 3-12 Sources of nutrition during the month prior to the baseline, 3 month and 12 month study visits, where sources are not mutually exclusive

Detailed dietary intake data were available for 79, 66 and 57 patients at baseline, 3- and 12 months respectively. The contribution of food, ONS and enteral formulas at baseline, 3- and 12 months to mean daily energy and protein intakes are shown in Figure 3-13. There was 1 patient at 3 months whose only source of nutrition was parenteral nutrition (i.e. nothing by the oral/enteral route), who was not included in the 3-month analysis.

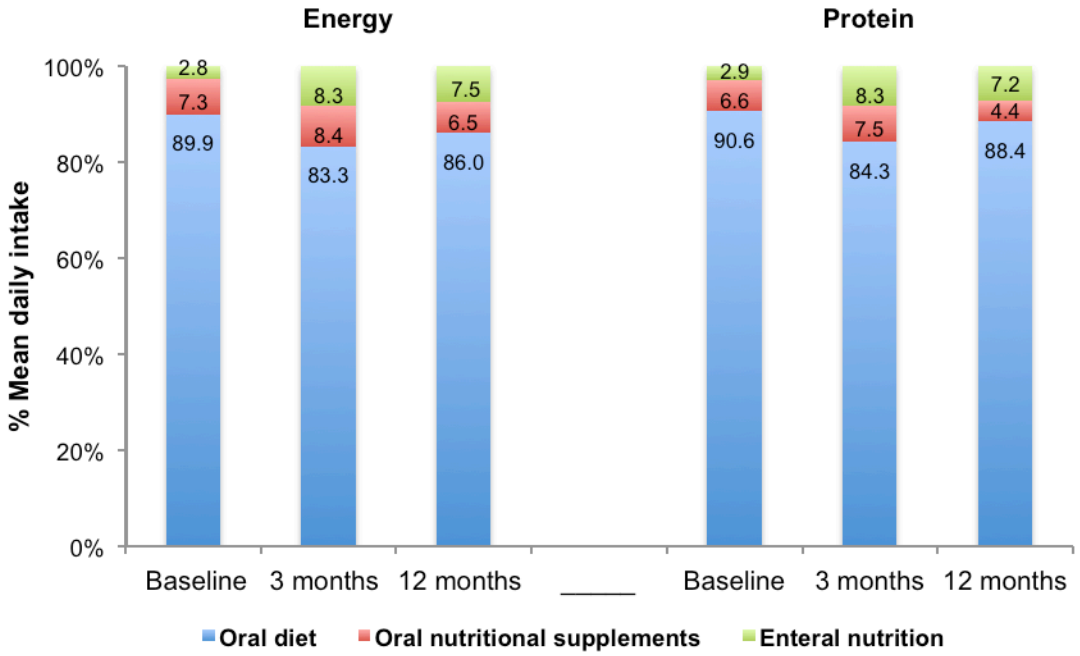


Figure 3-13 Mean contributions of food, oral nutritional supplements and enteral formulas to mean daily energy (kcal) and protein (g) intake at baseline (n= 79), 3 months (n= 66) and 12 months (n= 57)

There were 78, 61 and 53 FFQs analysed respectively. The reasons for the 13 unavailable FFQs were: (a) questionnaire not complete at visit for 1 patient at baseline; (b) incomplete questionnaire for 1 patient at 3 months; (c) no food being eaten for 1 patient at baseline, 6 patients at 3 months and 4 patients at 12 months. For the patients who completed the FFQs, the mean (SD) daily intake of energy, fibre, 24 nutrients and 14 food groups from food at each study visit are reported in Table 3-14.

Table 3-14 Mean (SD) daily intake of energy, macronutrients, fibre, micronutrients and food groups from food alone at baseline, 3 months and 12 months

	Baseline, n= 78	3 months, n= 61	12 months, n= 53
	Mean (SD)	Mean (SD)	Mean (SD)
Energy, kcal/d	2226.3 (1567.2)	2095.3 (873.6)	2112.3 (836.4)
Protein, g/d	90.2 (57.0)	86.1 (32.5)	85.7 (28.5)
Carbohydrate, g/d	268.6 (208.8)	242.2 (101.9)	243.1 (106.7)
Fat- total, g/d	88.0 (66.6)	89.7 (43.6)	90.8 (42.8)
Alcohol, g/d	9.7 (17.0)	5.3 (11.1)	6.1 (9.8)
Englyst Fibre, g/d	18.0 (15.5)	15.3 (7.4)	15.2 (7.8)
Micronutrients- vitamins			
Vitamin A, µg/d	1693.2 (1333.0)	1776.9 (1673.5)	1715.3 (1288.1)
Vitamin B_1, mg/d	1.7 (1.2)	1.5 (0.6)	1.5 (0.6)
Vitamin B_2, mg/d	2.4 (1.4)	2.2 (0.9)	2.2 (1.0)
Vitamin B_3, mg/d	23.6 (14.7)	22.1 (9.0)	22.2 (8.6)
Vitamin B_6, mg/d	2.4 (1.5)	2.1 (0.8)	2.1 (0.7)
Vitamin B_{12}, µg/d	8.3 (5.5)	9.1 (7.4)	8.3 (4.5)
Carotene, mg/d	4.1 (4.2)	3.8 (2.2)	3.3 (2.1)
Vitamin C, mg/d	118.8 (80.4)	113.7 (61.2)	109.5 (69.7)
Vitamin D, µg/d	3.5 (3.4)	3.5 (1.8)	3.4 (2.1)
Vitamin E, mg/d	13.3 (11.4)	12.2 (5.8)	12.5 (6.5)
Folate, µg/d	332.5 (213.8)	296.7 (126.7)	292.1 (122.8)
Micronutrients- minerals			
Calcium, mg/d	1131.1 (723.7)	1052.2 (389.5)	1015.3 (444.9)
Chloride, mg/d	4950.7 (3513.6)	4721.1 (1901.8)	4370.4 (1844.2)
Iron, g/d	12.3 (8.6)	11.0 (4.9)	11.1 (4.4)
Magnesium, mg/d	343.8 (217.9)	308.7 (119.4)	307.0 (118.5)
Phosphorus, mg/d	1598.1 (1011.9)	1509.5 (534.5)	1480.9 (505.1)
Potassium, mg/d	3943.4 (2454.2)	3534.4 (1245.1)	3492.2 (1242.5)
Selenium, µg/d	67.1 (43.2)	67.4 (33.7)	64.5 (25.6)
Sodium, mg/d	3337.6 (2469.9)	3179.8 (1298.3)	2947.6 (1258.0)
Zinc, mg/d	10.4 (6.8)	9.8 (3.9)	9.6 (3.2)
Food groups			
Alcoholic beverages, g/d	174.6 (371.7)	111.0 (263.3)	100.8 (189.6)
Cereals, cereal products, g/d	278.4 (236.4)	238.0 (125.4)	243.2 (151.3)
Eggs, egg dishes, g/d	24.4 (27.5)	22.8 (18.3)	20.1 (21.7)
Fats, oils, g/d	26.1 (25.0)	28.0 (20.9)	28.6 (24.4)
Fish, fish products, g/d	45.0 (39.4)	53.0 (58.7)	49.1 (42.3)
Fruit, g/d	213.6 (235.0)	146.2 (122.1)	98.2 (223.3)
Meat, meat products, g/d	102.2 (78.3)	113.4 (87.6)	114.5 (63.5)
Milk, milk products, g/d	467.3 (324.8)	442.7 (224.0)	413.4 (236.2)
Non-alcoholic beverages, g/d	937.7 (575.2)	894.2 (484.5)	799.9 (405.3)
Nuts, seeds, g/d	3.5 (7.2)	4.6 (8.1)	11.5 (27.6)
Potatoes, g/d	95.1 (73.5)	87.1 (84.0)	77.3 (47.9)
Soups, sauces, g/d	149.3 (185.0)	112.7 (84.0)	101.6 (150.2)
Sugars: preserves, snacks, g/d	67.5 (76.1)	64.2 (55.7)	59.9 (45.9)
Vegetables, g/d	301.7 (314.2)	254.6 (163.8)	230.5 (151.8)
Note: Vitamin A refers to retinol equivalents; Carotene refers to total carotene equivalents			

There were 43 patients who completed a FFQ at each of the three study visits. The mean (SD) weight for these patients was 75.8 (14.1) at baseline, 74.2 (13) at 3 months and 70.4 (12.3) at 12 months. This weight change was significantly lower at (a) 3 months compared with baseline (p= 0.022), (b) 12 months compared with baseline (p< 0.0001) and (c) 12 months compared with 3 months (p= 0.002) (i.e. paired score comparisons). This significant weight loss is of relevance for the interpretation of the dietary assessment results in this group providing data at each time point. For these 43 patients, the percentage energy provided by protein, carbohydrate, fat and alcohol at each study visit is shown in Figure 3-14. Results for the comparison of mean (SD) daily energy, fibre, nutrient and food group intakes at each visit, for these 43 patients, are shown in Table 3-15. There was no significant change in the intake of any of the variables over time following Bonferroni post-hoc testing.

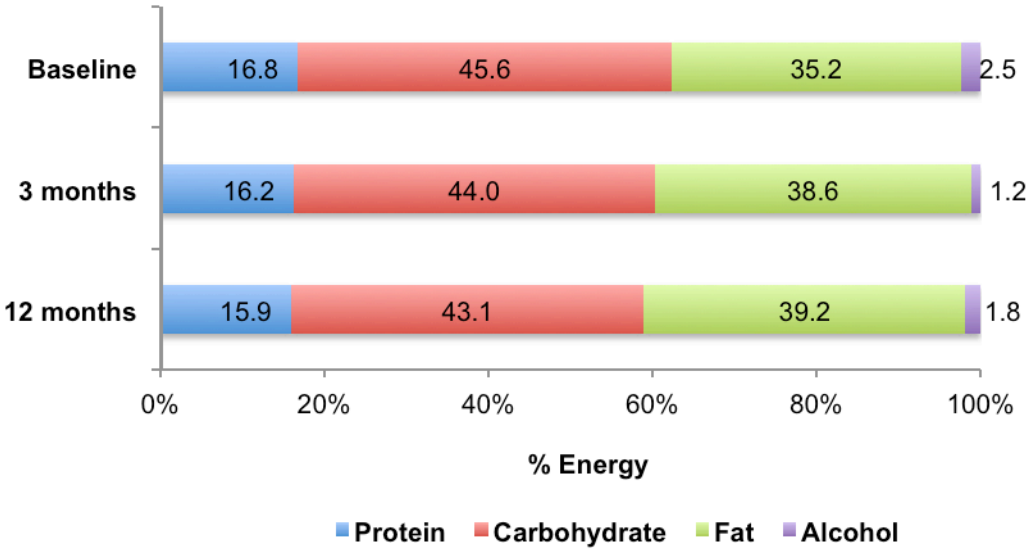


Figure 3-14 Percentage energy provide by protein, carbohydrate, fat and alcohol from food at each study visit for the 43 patients with 3 food frequency questionnaires

Table 3-15 Comparison of mean daily intake of energy, fibre, nutrients and food groups from food at each study visit in those 43 patients providing data at all 3 time points

n= 43	Baseline	3 months	12 months	p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
Energy, kcal/d	2253.1 (1179.5)	2222.1 (957.4)	2162.8 (834.6)	0.869
Protein, g/d	94.5 (46.1)	90.3 (42.8)	86.1 (29.8)	0.505
Carbohydrate, g/d	273.8 (157.7)	261.0 (110.4)	248.6 (102.6)	0.583
Fat- total, g/d	88.2 (52.5)	95.2 (47.5)	94.3 (43.7)	0.684
Alcohol, g/d	7.9 (13.3)	3.7 (6.8)	5.7 (9.6)	0.022 *
Englyst Fibre, g/d	18.9 (12.5)	17.0 (8.9)	15.6 (7.8)	0.191
Micronutrients- vitamins				
Vitamin A, µg/d	1936.5 (1149.1)	1997.6 (1827.2)	1730.4 (1204.4)	0.609
Vitamin B_1, mg/d	1.7 (1.0)	1.6 (0.8)	1.5 (0.6)	0.239
Vitamin B_2, mg/d	2.5 (1.2)	2.4 (1.1)	2.2 (1.0)	0.458
Vitamin B_3, mg/d	24.4 (11.7)	23.0 (10.2)	22.0 (9.2)	0.502
Vitamin B_6, mg/d	2.5 (1.2)	2.3 (1.1)	2.1 (0.8)	0.157
Vitamin B_{12}, µg/d	9.1 (4.9)	9.2 (6.7)	8.3 (4.6)	0.664
Carotene, mg/d	4.3 (3.4)	4.1 (2.4)	3.3 (2)	0.099
Vitamin C, mg/d	131.1 (78.2)	130.5 (80.3)	113.6 (73.6)	0.165
Vitamin D, µg/d	3.7 (2.8)	3.8 (1.8)	3.6 (2.3)	0.870
Vitamin E, mg/d	14.3 (9.3)	13.2 (5.9)	12.8 (6.7)	0.586
Folate, µg/d	351.7 (178.5)	329.5 (168.5)	296.8 (129.7)	0.125
Micronutrients- minerals				
Calcium, mg/d	1181.1 (609.5)	1144.0 (542.1)	1058.3 (439.7)	0.297
Chloride, mg/d	4874.0 (2720.9)	4773.8 (1649.6)	4348.0 (1705.6)	0.369
Iron, g/d	12.6 (6.5)	11.3 (4.3)	11.2 (4.5)	0.282
Magnesium, mg/d	362.9 (182.3)	331.3 (169.1)	315.6 (123.5)	0.204
Phosphorus, mg/d	1665.7 (809.1)	1598.5 (673.8)	1511.3 (516.5)	0.416
Potassium, mg/d	4177.6 (1934.9)	3773.1 (1581.0)	3548.6 (1269.1)	0.079
Selenium, µg/d	72.3 (33.6)	69.5 (32.2)	63.6 (25.7)	0.253
Sodium, mg/d	3277.3 (1905.6)	3235.6 (1175.8)	2930.8 (1180.2)	0.395
Zinc, mg/d	10.8 (5.8)	10.3 (5.7)	9.8 (3.3)	0.488
Food groups				
Alcoholic beverages, g/d	138.9 (273.6)	70.0 (138.7)	106.9 (205.9)	0.041 *
Cereals, cereal products, g/d	270.1 (183.6)	257.9 (130.1)	246.3 (139.9)	0.731
Eggs, egg dishes, g/d	23.4 (25.2)	22.0 (14.8)	20.5 (22.8)	0.739
Fats, oils, g/d	28.0 (21.6)	30.7 (21.6)	30.4 (24.6)	0.726
Fish, fish products, g/d	49.2 (35.0)	46.6 (32.7)	43.1 (35.9)	0.648
Fruit, g/d	241.3 (261.9)	171.0 (184.0)	204.5 (210.8)	0.124
Meat, meat products, g/d	103.2 (65.4)	113.7 (82.3)	110.5 (63.5)	0.722
Milk, milk products, g/d	485.5 (272.7)	454.5 (206.3)	435.0 (225.9)	0.485
Non-alcoholic beverages, g/d	1032.5 (532.5)	932.4 (471.1)	817.5 (406.4)	0.052
Nuts, seeds, g/d	3.9 (6.4)	4.9 (8.3)	13.7 (30.2)	0.040 *
Potatoes, g/d	100.2 (61.5)	89.1 (50.2)	74.3 (48.8)	0.062
Soups, sauces, g/d	130.9 (157.1)	109.0 (93.9)	76.9 (67.5)	0.076
Sugars: preserves/snacks, g/d	63.5 (55.4)	64.2 (60.0)	65.7 (47.8)	0.979
Vegetables, g/d	319.9 (257.9)	284.0 (175.6)	238.2 (156.5)	0.108

Notes: Vitamin A refers to retinol equivalents; Carotene refers to total carotene equivalents
Repeated measures ANOVA with Mauchly's Test of Sphericity was performed: Greenhouse-Geisser correction was made if the data violated the assumption of sphericity.
For nutrient/food groups with significantly different means (* $p < 0.05$) using ANOVA, a Bonferroni post-hoc test was performed to determine which specific means differed.
There was found to be no significant difference between any of the 3 study visits for alcohol, alcoholic beverages and nuts, seeds after post-hoc testing.

Analysis was performed to determine the proportion of patients meeting EAR for energy and RNI for protein, fibre and micronutrients from (a) food and (b) food, ONS and EN combined. Estimated average requirement for energy was met by ≤ 50% at all study visits (Table 3-16, orange and red shading). The RNIs for magnesium, potassium, selenium and zinc consistently failed to be met by at least 25% of patients, with proportions particularly low for potassium and selenium (orange and red shading). Food alone was not adequate in meeting the RNIs for iron in 28.3-32.8% of cases, while for calcium, only one study visit saw > 75% of patients achieving their RNI from food. Throughout the study duration, < 34% of the cohort met their RNI for fibre, while < 16% of those aged 65 or more met their RNI for vitamin D (a RNI for vitamin D is only available for those aged > 65 years).

Table 3-16 Proportion of patients meeting EAR for energy and RNI for protein, fibre and micronutrients as provided by (a) food alone and (b) food, oral nutritional supplements and enteral nutrition combined

	Meeting requirement at baseline, n (%)		Meeting requirement at 3 months, n (%)		Meeting requirement at 12 months, n (%)	
	Food alone, n= 78	Food, ONS and EN, n= 79	Food alone, n= 61	Food, ONS and EN, n= 66	Food alone, n= 53	Food, ONS and EN, n= 57
Energy	29 (37.2)	34 (43.0)	23 (37.7)	33 (50.0)	22 (41.5)	28 (49.1)
Protein	62 (79.5)	67 (84.8)	54 (88.5)	61 (92.4)	48 (90.6)	52 (91.2)
Englyst Fibre	26 (33.3)	26 (32.9)	17 (27.9)	17 (25.8)	12 (22.6)	14 (24.6)
Micronutrients- vitamins						
Vitamin A	63 (80.8)	69 (87.3)	52 (85.2)	59 (89.4)	47 (88.7)	51 (89.5)
Vitamin B_1	66 (84.6)	72 (91.1)	54 (88.5)	61 (92.4)	45 (84.9)	52 (91.2)
Vitamin B_2	65 (83.3)	70 (88.6)	52 (85.2)	60 (90.9)	46 (86.8)	51 (89.5)
Vitamin B_3	61 (78.2)	66 (83.5)	50 (82.0)	60 (90.9)	42 (79.2)	50 (87.7)
Vitamin B_6	65 (83.3)	72 (91.1)	53 (86.9)	60 (90.9)	45 (84.9)	50 (87.7)
Vitamin B_{12}	74 (94.9)	77 (97.5)	60 (98.4)	65 (98.5)	52 (98.1)	56 (98.2)
Vitamin C	67 (85.9)	73 (92.4)	55 (90.2)	62 (93.9)	49 (92.5)	54 (94.7)
Vitamin D *	2 (3.1)	3 (6.4)	0 (0)	6 (15)	0 (0)	4 (13.3)
Folate	65 (83.3)	69 (87.3)	51 (83.6)	58 (87.9)	42 (79.2)	50 (87.7)
Micronutrients- minerals						
Calcium	58 (74.4)	63 (79.7)	48 (78.7)	57 (86.4)	39 (73.6)	43 (75.4)
Chloride	68 (87.2)	69 (87.3)	56 (91.8)	58 (87.9)	45 (84.9)	47 (82.5)
Iron	54 (69.2)	54 (68.4)	41 (67.2)	41 (62.1)	38 (71.7)	47(82.5)
Magnesium	47 (60.3)	52 (65.8)	35 (57.4)	46 (69.7)	27 (50.9)	32 (56.1)
Phosphorus	75 (96.2)	77 (97.5)	60 (98.4)	64 (97.0)	53 (100)	55 (96.5)
Potassium	44 (56.4)	47 (59.5)	29 (47.5)	36 (54.5)	20 (37.7)	22 (38.6)
Selenium	30 (38.5)	38 (48.1)	27 (44.3)	41 (62.1)	22 (41.5)	29 (50.9)
Sodium	68 (87.2)	69 (87.3)	56 (91.8)	59 (89.4)	46 (86.8)	50 (87.7)
Zinc	45 (57.7)	54 (68.4)	34 (55.7)	47 (71.2)	30 (56.6)	41 (71.9)

Abbreviations: EN, enteral nutrition; ONS, oral nutritional supplements
Notes: Energy and protein intakes are based on average daily intakes.
Vitamin A refers to retinol equivalents.
* RNI for vitamin D only available for those aged 65+ years: there were 47, 38 and 28 patients providing data for food alone, at baseline, 3- and 12 months; there were 47, 40 and 30 patients providing data for food, ONS and enteral formulas respectively.
Key:
Red= < 50% of patients were meeting dietary reference value
Orange= ≥ 50 - < 75% of patients were meeting dietary reference value
Green= ≥ 75% of patients were meeting dietary reference value

Further analysis was performed to determine the proportion of patients meeting EAR for energy and RNI for protein from food alone, according to GSRS total score and SGA category at each study visit (Table 3-17). Median GSRS total score was lower (fewer/less severe symptoms) for those not meeting energy and protein requirements as compared with those meeting requirements, with the exception of protein at 3 months. There were higher proportions of patients not meeting energy requirements that were considered malnourished (SGA B+C), as compared with those meeting requirements, at each of the three time points.

Table 3-17 Median (range) Gastrointestinal Symptom Rating Scale total score and Subjective Global Assessment category for patients meeting and not meeting EAR for energy and RNI for protein from food

	Energy		Protein	
	Meeting Requirements	Not meeting Requirements	Meeting Requirements	Not meeting Requirements
Gastrointestinal Symptom Rating Scale				
Total Score	***Baseline, n= 78***			
	n= 29	n= 49	n= 62	n= 16
Median (range)	15 (0-38)	10 (0-46)	12 (0-39)	11.5 (2-46)
	3 months, n= 61			
	n= 23	n= 38	n= 54	n= 7
Median (range)	12 (0-28)	8 (0-39)	9 (0-39)	15 (4-24)
	12 months, n= 53			
	n= 22	n= 31	n= 48	n= 5
Median (range)	13.5 (4-46)	9 (0-37)	13 (0-46)	5 (1-22)
Subjective Global Assessment				
Category	***Baseline, n (%)***			
SGA A, n= 31	10 (32.3)	21 (67.7)	27 (87.1)	4 (12.9)
SGA B+C, n= 47	19 (40.4)	28 (59.6)	35 (74.5)	12 (25.5)
	3 months, n (%)			
SGA A, n= 23	11 (47.8)	12 (52.2)	23 (100)	0 (0)
SGA B+C, n= 38	12 (31.6)	26 (68.4)	31 (81.6)	7 (18.4)
	12 months, n (%)			
SGA A, n= 22	9 (40.9)	13 (59.1)	20 (90.9)	2 (9.1)
SGA B+C, n= 31	13 (41.9)	18 (58.1)	28 (90.3)	3 (9.7)
Notes: Energy and protein intakes are based on average daily intakes from food. SGA A= well-nourished, SGA B= moderately/suspected malnourished, SGA C= severely malnourished				

3.5.9 Small Intestinal Bacterial Overgrowth

Throughout the study duration, there was a total of 38 GHMBTs performed in a sub-group of 17 (21.3%) patients. All 17 underwent a baseline test, of which 4 (23.5%) were positive for SIBO, none of whom opted for treatment at that point. At 3 months, the same 17 underwent a second GHMBT, of which 7 (7/13= 53.8%) had a new positive result. At 12 months, of the only four who underwent a third test, 3 (3/4= 75%) had a new positive result (Figure 3-15). In total there were 4 (10.5%) incomplete tests (i.e. the test was persistently negative but was not continued until 180 minutes at the patient's request). Using both complete and incomplete tests, the prevalence of SIBO at baseline was 23.5% (4/17) and its incidence after baseline was 76.9% (10/13). Therefore, the overall SIBO diagnosis at baseline and subsequent time points was 14/17 (82.4% with 95% CI: 58-95%).

All but 4 (28.6%) of these 14 correctly adhering to the pre-test guidelines described in Section 2.4.1.2; 3 (21.4%) ate slowly absorbed carbohydrates during the previous 24 hours, while 1 (7.2%) did not brush teeth or use mouthwash on the morning of the test. Of note, the three who ate slowly absorbed carbohydrates did not have raised basal H_2 levels. As such, the non-adherence to the diet did not cause a false positive at the basal measurement.

Of the positive tests, 3 (21.4%) were positive for CH_4 alone, 11 (78.6%) were positive for both gases and none were positive for H_2 alone. The characteristics and treatment modalities of those with SIBO and those without are reported in Table 3-18.

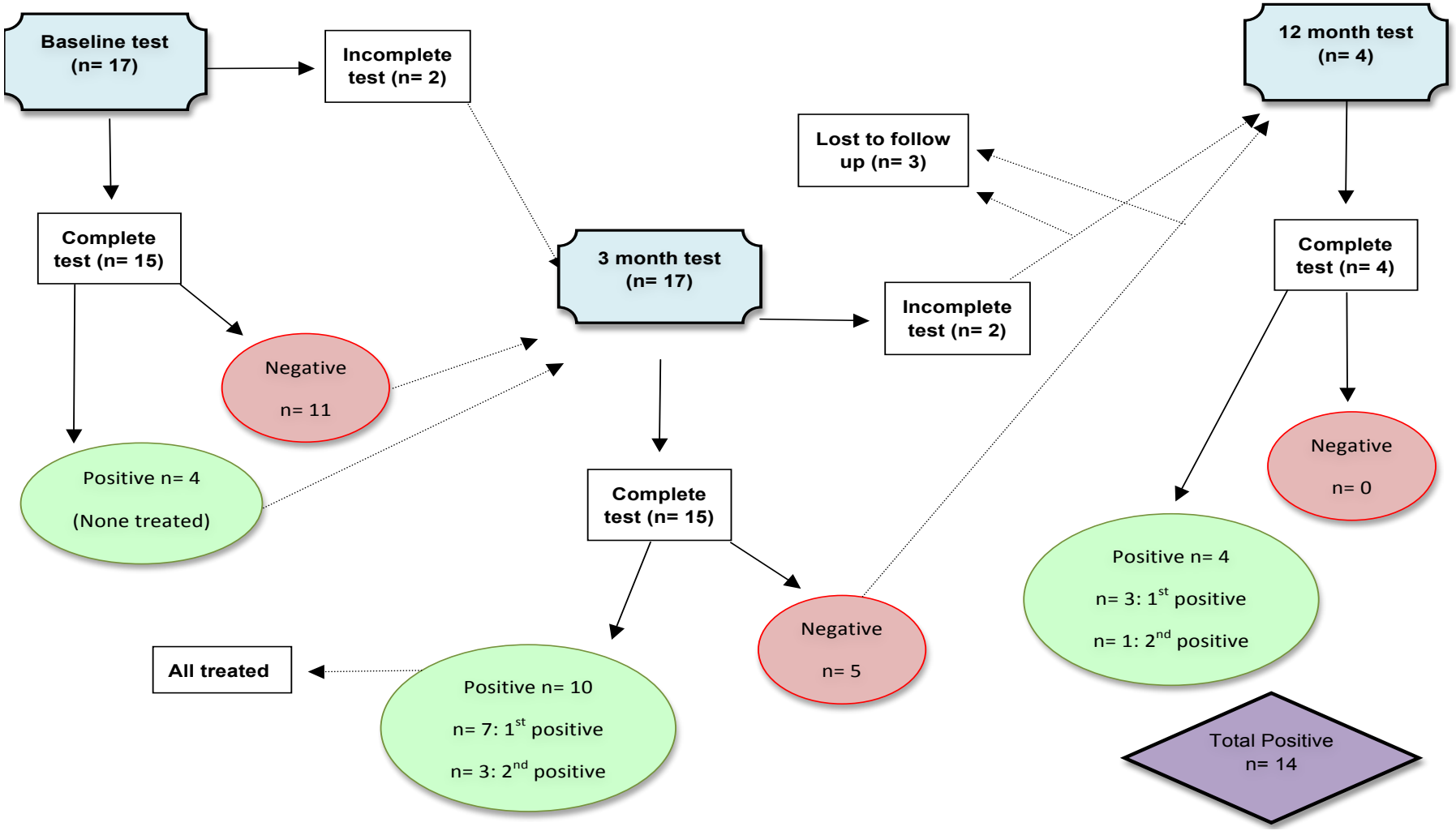


Figure 3-15 Glucose hydrogen methane breath test results in the sub-group of 17 patients who underwent testing

Table 3-18 Characteristics, gastrointestinal symptoms and treatment modalities of patients testing positive for small intestinal bacterial overgrowth using the glucose hydrogen methane breath test compared with those testing negative for it

	SIBO Positive, n= 14	SIBO Negative, n= 3
Demographics, characteristics and predisposing conditions for SIBO		
Male, n (%)	13 (92.8)	1 (33.3)
Age in years, mean (SD)	64. 8 (8.4)	57 (10.1)
Weight in kg, mean (SD)	74.1 (16.4)	62 (19.9)
SGA B or C, n (%)	11 (78.6)	3 (100)
IBS, n (%)	2 (14.3)	0 (0)
Diabetes mellitus, n (%)	2 (14.3)	0 (0)
Previous GI surgery, n (%)	2 (14.3)	0 (0)
Medications in 1 month before testing: n (%)		
Antibiotics	1 (7.1)	0 (0)
Antisecretory, pro-/antimotility agents	7 (50)	2 (66.6)
Laxatives, stool softeners	3 (21.4)	1 (33.3)
Gastrointestinal Symptom Rating Scale (GSRS) symptoms: n (%)		
Dysphagia to solids	5 (35.7)	1 (33.3)
Dysphagia to fluids	3 (21.4)	1 (33.3)
Odynophagia to solids	1 (7.1)	0 (0)
Odynophagia to fluids	0 (0)	0 (0)
Regurgitation of solids	4 (28.6)	1 (33.3)
Regurgitation of fluids	4 (28.6)	1 (33.3)
Heartburn	2 (14.3)	1 (33.3)
Acid reflux	5 (35.7)	0 (0)
Belching	9 (64.3)	3 (100)
Nausea	5 (35.7)	1 (33.3)
Early satiety	6 (42.9)	1 (33.3)
Bloating	0 (0)	1 (33.3)
Abdominal grumbling	6 (42.9)	1 (33.3)
Abdominal pain	6 (42.9)	1 (33.3)
Flatulence	10 (71.4)	2 (66.7)
Loose stools	10 (71.4)	0 (0)
Diarrhoea	8 (57.1)	0 (0)
Faecal urgency	4 (28.6)	2 (66.7)
Faecal incontinence	2 (14.3)	0 (0)
Hard stools	7 (50)	0 (0)
Constipation	6 (42.9)	0 (0)
Incomplete evacuation	3 (21.4)	2 (66.7)
GSRS total scores, median (range)	8.5 (2-46)	7 (4-13)
Oncological treatment received: n (%)		
	SIBO Positive, n= 10 *	**SIBO Negative, n= 3**
Surgery	5 (50)	1 (33.3)
Radiotherapy	8 (80)	3 (100)
Chemotherapy	9 (90)	3 (100)
Notes: Symptoms may be mild, moderate or severe. For those with a positive test, the symptoms are reported for the 1 month before test positivity. For those with all negative tests, the symptoms are reported for the 1 month before the 12 month time point. * The n= 4 patients diagnosed at baseline were not included as they had not yet received any oncological treatment		

3.6 Discussion

3.6.1 Gastrointestinal Symptoms and Nutritional Status

In order to address GI and nutritional status deterioration in OG cancer, it is important to gather objective data on their evolution throughout the course of the disease. This is the first large prospective study to systematically record GI symptoms and nutritional status in OG cancer during the first year after diagnosis. As hypothesised, OG cancer and/or radical treatment cause the persistence or development of GI symptoms and malnutrition: in those assessed before treatment and 12 months later, moderate-severe GI symptoms and malnutrition persisted or developed in 71.9% and 59.7% respectively. There was an improvement seen in GI symptoms and nutritional status in less than one-third: 28.1% of patients went form having moderate-severe GSRS symptoms at baseline to none-mild symptoms at 12 months; 22.8% went from being malnourished to being well-nourished. Thus, it can be said that GI symptoms and malnutrition are persistent during this first year post-diagnosis in the majority of patients with OG cancer and Hypotheses 1 and 2 were accepted.

It is evident that the GI symptom burden of OG cancer patients is great. In fact, the prevalence of many symptoms was higher in the current cohort than has been reported for other OG cancer cohorts. For example, the prevalence of diarrhoea 12 months after oesophagectomy and 6-66 months after gastrectomy was previously reported as 8% and 28% respectively (Mine et al. 2010; Ludwig et al. 2001). The present study found higher diarrhoea prevalence rates: 41% and 46% of patients had it at 3- and 12 months, with the majority of the cohort having undergone surgery by the latter time point. Likewise for nausea, other researchers have described its presence in 20-30% and 19-26% of patients at 6- and 12 months after surgery for OG cancer respectively (Ludwig et al. 2001; Ginex et al. 2013; Mine et al. 2010). However, in the current study, a higher percentage of patients reported feeling nauseous: 54% at 3 months and 38% at 12 months. As a final example, abdominal pain was more frequently reported in the current cohort at 12 months (60%) as compared with the literature, where the symptom prevalence is said to be between 10% and 47% depending on the time since surgery (Ginex et al. 2013; Visick 1948; Olsson et al. 2007; Mine et al. 2010). As previously discussed in Section 1.1.3,

GSRS symptom scores were shown to be significant in explaining 50.3% of variance in QoL ($F= 13.646$; $p< 0.001$). Considering the results in the current cohort, it is apparent that more emphasis needs to be placed on the optimisation of GI function during treatment. In doing so, QoL may too be improved, although this was not measured here.

With regard to malnutrition, cancers of the GI tract are known to produce higher nutritional risk than other cancer sites (Koom et al. 2012; Hebuterne et al. 2014; Baldwin et al. 2006). In fact, Heburterne et al.'s prevalence study (using the NRI screening tool) indicated that patients with OG cancer had the second highest prevalence of malnutrition (60%) after pancreatic cancer patients (67%) in a cohort that included patients with a variety of cancer diagnoses. This supports earlier work where 61% of newly diagnosed OG cancer patients were shown to have > 5% unintentional weight loss (Baldwin et al. 2006). These preceding malnutrition risk data for OG cancer (60-61%) are very similar to those in the current study (59.6-61.8% were found to be malnourished).

Although this high prevalence of malnutrition is unsurprising, it is nonetheless alarming, considering this is a group undergoing radical treatment, which often necessitates multiple hospital attendances every month, and therefore, many interactions with healthcare professionals. Notably, 40%, 66% and 74% of the cohort had at least one dietetic consultation in the period before the baseline, 3 month and 12 month assessment respectively. As such, the majority of those followed-up after baseline had contact with a dietitan. However, it appears that the dietetic involvement was not sufficient, given that 26.4% became malnourished, while 33.3% maintained their malnourished status during the study, suggesting that malnutrition was not effectively treated in this majority. However, it should be mentioned that 17.5% were consistently well-nourished and 22.8% improved and became well-nourished, suggesting effective preservation or realisation of good nutritional status in 40.3%. Importantly, just 57 of the initial 80 patients completed a 12 month assessment, and therefore, the true number of malnourished and well-nourished patients may have differed had complete data been obtained for those who withdrew or were lost to follow-up.

The consequences of malnutrition in this setting are well established. However, measuring the consequences of malnutrition was not within the scope of this thesis and so one can only speculate as to whether malnutrition contributed towards increased toxicity to oncological treatments, lower response to treatment, deteriorating performance status, adverse effects on immunity, longer length of stay in hospital, poorer QoL and/or lower overall survival (van Cutsem & Arends 2005; Andreyev et al. 1998; Dewys et al. 1980; Hebuterne et al. 2014; Kyle et al. 2005; Lis et al. 2012). Of interest, the treatment modalities intended for patients at baseline differed from the actual treatment received by 12 months, with fewer patients undergoing surgery than was originally planned. The reason for deviation from the original treatment plan was not formally recorded in this study (though the MDT kept records), but the research dietitian noted cases where the patient's performance status worsened and as a result, they were no longer deemed fit for surgery.

With regard to the third study hypothesis, the data suggests that there is an imperfect positive relationship between GSRS total scores and PG-SGA total scores at all time points. Thus, Hypothesis 3 was accepted. In effect, high symptom burden tends to be associated with poorer nutritional status and low symptom burden tends to be associated with better nutritional status. A moderate correlation was found at all study time points and was strongest at baseline (r= +0.55). As such, a change in one variable at diagnosis was more likely to be accompanied by a change in the other variable at baseline, when compared with the 3- and 12 month points (where r= +0.51 and +0.42 respectively). This is supported by the baseline finding that 11 GI symptoms were significantly associated with malnutrition (using SGA), but at 3 months significant associations were seen for just three symptoms and at 12 months there were no significant associations.

There has been previous research investigating the relationship between symptoms and nutritional status in cancer, though in most cases very few GI symptoms were included in the analysis and the assessment of nutritional status was based solely on unintentional weight loss rather than PG-SGA result. Also, there have been no studies specific to OG cancer.

Nevertheless, in various cancer cohorts, it has been demonstrated that dysphagia, nausea, bloating and early satiety are significantly associated with weight loss of varying degrees (Sánchez-Lara et al. 2012; Bovio et al. 2009; Khalid et al. 2007; Chate 2006; Petruson et al. 2005; Grosvenor et al. 1989). This agrees with the findings from the current study, where dysphagia and nausea were found to be significantly associated with SGA category at baseline. Also, early satiety was significantly associated with SGA category at baseline and 3 months and percentage weight loss at 3 months. Bloating was not associated with SGA at any time point, but an associated with percentage weight loss was noted at 3 months.

While a moderate relationship between symptoms and nutritional status was demonstrated, one cannot claim a cause and effect relationship. Many factors may be involved in the development of malnutrition including disease-related anorexia, increased macronutrient requirements and catabolic factors, rather than just the presence of GI symptoms. Likewise, numerous factors may be involved in the development of GI symptoms including treatment toxicity, motility irregularities, hormonal abnormalities, pharmacological agents and SIBO rather than just poor nutritional status. These confounding factors would need to be controlled for before a causal relationship could be established. In addition, many other important factors need to be considered before a causal inference could be made. For example, the cause (independent variable) must be shown to precede the effect (dependent variable) i.e. the presence of GI symptoms must have developed before the individual became malnourished. Also, a dose-response relationship must be confirmed (i.e. the more severe the GI symptoms, the worse the nutritional status) as well as reversible association (i.e. the resolution of the GI symptoms should improve nutritional status). Furthermore, multiple studies in different geographical locations should produce similar effects before a cause and effect relationship can be determined, with biological plausibility also required (i.e. a robust biological mechanism for the development of malnutrition as a direct result of GI symptoms). One could speculate that an intervention to manage GI symptoms may positively influence nutritional status, but because of the difficulty in controlling for confounding factors, one may never be able to establish that the GI intervention was the sole cause for improved nutritional status. Nonetheless, even without

establishing a relationship, the intervention may still be effective, and this warrants future prospective research.

3.6.1.1 Strengths and Limitations of Gastrointestinal Symptoms and Nutritional Status Assessment Methods and Results

This study was designed to measure GI symptoms and nutritional status acutely and chronically, with the hope that the 12 month (chronic) time point would capture the patient's status after the completion of treatment. Normally, in patients treated with curative intent, radical treatment is finished within 12 months and so this was chosen as the final time point. However, in this cohort, 29.8% of patients were still undergoing or planned for further oncological treatment at 12 months, often due to treatment delays. Therefore, for a minority of the cohort, their 12 month data is not a true reflection of the *post*-treatment period. If time were not limited, an additional two study visits at 18- and 24 months would have provided interesting data with regard to the actual chronic GI symptoms and nutritional status in this cohort, but practical issues of long term follow-up in the confines of a PhD prevented this from being possible.

As with any longitudinal study, particularly involving patients with cancer, attrition due to the death and decline of patients is inevitable. There were 14 (17.5%) patients who died during the study, 6 (7.5%) who withdrew before 12 months and a further 3 (3.8%) who were lost to follow-up at 12 months. Thus, 23 (28.8%) patients did not complete the study. It is likely that this group differed from those who completed the study, as many patients reported feeling too unwell or fatigued to complete the study assessments and so withdrew themselves or were lost to follow-up, and of course those who died may themselves have had worse GI symptoms and nutritional status than the surviving patients. Therefore, the remaining group may not be representative of the original cohort and may offer a superior picture than is true with regard to GI symptoms and nutritional status.

Conversely, sampling bias does not appear to be relevant in this study: the recruited population and the declined population were very similar with regard to gender spread, age, race and

performance status. As such, a systematic error caused by non-random sampling does not appear important here.

It must be borne in mind that the content of the GSRS used in this study was the result of a multi-professional discussion. Obviously, the 22 symptoms included are not the only possible symptoms that a patient with OG cancer can experience. For example, vomiting, steatorrhoea and nocturnal defaecation were not included in the tool, though they may be relevant to this cohort. Consequently, this study may have underestimated the real symptom burden. This problem could have been minimised had the (modified) GSRS been validated (the process by which a questionnaire is assessed for its accuracy and reliability) for use in OG cancer patients undergoing multimodality treatment. However, this was not achievable due to time constraints. As it was not validated, one cannot be completely certain of the trustworthiness of the data produced (i.e. whether it has good reliability and validity). For example, when the reliability is not known, the degree to which the questionnaire will produce the same result if completed by the same patient, under the same conditions, more than once, is not known. Also, it is not possible to determine if the results of this study can be generalised beyond the current RM cohort, as we do not know its external validity. Despite these limitations, this is the first prospective study to measure a large number of both upper- and lower-GI symptoms in an OG cancer cohort over a period of one year. Thus, the symptom data presented will be a valuable contribution to the literature in this area.

Interestingly, after the commencement of the current study, the Dysfunction After Upper Gastrointestinal Surgery (DAUGS20) tool was validated for use in patients with OG cancer (Nakamura et al. 2011). This is a 20-item objective evaluation tool designed to evaluate postoperative symptoms. Construct validity (i.e. how well the scale measures the construct it was designed to measure) was confirmed using the *'known-group'* method. The DAUGS20 tool, if available when the current study was being designed, would have represented an acceptable alternative to the GSRS. Still, given that it was not validated for use in patients undergoing

multimodality treatment, nor in cohorts outside of Japan, it would not have been without its limitations in the current study.

Another notable issue is that the current study did not measure the effect of the persistence or development of GI symptoms and malnutrition on QoL. The study dietitian did strongly consider measuring QoL in the study, but given the number of other questionnaires and components involved at each study visit, it was deemed too onerous to include another tool. It has been well established that QoL is compromised in patients with OG cancer at the time of diagnosis and also throughout the treatment pathway, particularly following surgery (Visser et al. 2006; Gillham et al. 2008; Viklund et al. 2006; Blazeby et al. 2000). Researchers have studied the correlation between nutritional status and QoL in patients with GI cancer for some time, with a 2012 systematic review of the literature (eight studies) concluding that better nutritional status was positively associated with better QoL (Lis et al.). However, to date, there has been little research focusing on the correlation between GI symptoms and QoL in this setting. Given the low five-year survival rate in this patient group, it is important to gain an understanding of the QoL effects in individuals with persistent GI symptoms. Future research in this area is warranted.

3.6.2 Nutrient and Food Group Intake Pattern

This is the first prospective study to measure the nutrient and food group intake pattern over the time course of treatment in OG cancer patients. When interpreting the data, it should be borne in mind that 39%, 24% and 32% of patients reported taking vitamin or mineral supplements at baseline, 3- and 12 months, and these were not included in the dietary analysis. The use of ONS and EN improved the cohort's ability to meet their requirements compared with food alone: 6%, 12% and 7% more patients met their EAR for energy; 5%, 7% and 4% more met their RNI for protein at baseline, 3- and 12 months respectively. This highlights the importance of nutritional support, particular at the acute time point. However, even with this nutritional support, only 43-50% of patients were meeting their EAR for energy at each time point. Given that there was no increase in energy intake during the course of the study, it is likely that the chronic

energy deficit contributed, at least in part, to the significant weight loss recorded during the study.

With respect to nutrients, vitamin D intake was inadequate in the over 65's of this cohort, which is not unexpected considering the high prevalence of vitamin D insufficiency and deficiency in the general UK population (Zgaga et al. 2011; Hyppönen & Power 2007). This high prevalence is related to the high northern latitude of the countries, which reduces individuals' exposure to the ultraviolet B wavelength necessary for vitamin D synthesis, thus leaving them reliant on exogenous sources of the vitamin (Zgaga et al. 2011; Pearce & Cheetham 2010). Also, it has previously been reported that serum levels of 25-hydroxyvitamin D are lower in gastrectomised patients compared with healthy controls (Zittel et al. 1997; Heiskanen et al. 2001). Vitamin D (in its active form, 1,25-dihydroxyvitamin D) plays an important role in bone metabolism as a calcium-regulating hormone, and so, optimising intake is essential for bone health. Unfortunately, metabolic bone disease is a well-known complication of gastrectomy, with data suggesting that bone resorption increases as early as one month after gastrectomy without a matching increase in bone formation (Baek et al. 2008; Zittel et al. 1997). The prevalence of secondary osteomalacia and osteoporosis in this group is reported to be as high as 55% (Lim et al. 2007; Zittel et al. 1997; Eddy 1971; Lim & Lee 2011).

As well as vitamin D, other minerals important for bone metabolism include calcium, magnesium, zinc and potassium. In the current study, with the exception of calcium, the intake of all of these minerals from food was poor: 50-75% of individuals met their RNIs for magnesium and zinc; 38-60% met their RNI for potassium. Although, there are likely to be many factors involved in the pathogenesis of osteoporosis in OG cancer including old age, female sex, low body weight, hormonal imbalances, malabsorption, use of proton pump inhibitors and treatment effects (National Institute of Health 2001), the current study provides evidence that mineral insufficiencies exist in this group and should be targeted to help reduce the risk of metabolic bone disease.

Iron is another mineral that has received a lot of attention in OG cancer, particularly in post-surgical patients, with 12%, 15% and 27-69% of individuals found to be iron deficient at one, two and three years after gastrectomy respectively (Lee et al. 2013; Lim 2012). Iron deficiency after gastrectomy is thought to be caused by decreased iron absorption due to reduced food intake and by-pass of the duodenum in some methods of reconstruction, as well as, reduced gastric acidity, which decreases the conversion of non-haem iron to the ferrous form, which is more absorbable (Lee et al. 2013). If a blind loop exists following surgery, SIBO may develop with subsequent ulceration and blood loss within the loop. This blood loss has been demonstrated in rats with experimental blind loops (Giannella & Toskes 1976). Also, in a cohort of patients post-gastrectomy, Brägelmann reported a tendency towards lower ferritin values and a higher frequency of positive faecal occult blood tests in patients with SIBO compared with those without SIBO (Brägelmann et al. 1997). Oral intake of iron has not been investigated previously in patients with OG cancer, but the current study indicates that intake is suboptimal with < 75% of patients meeting their RNI from food. The 12 month follow-up was the only time at which > 75% of the cohort met their RNI for iron from food, ONS and enteral formulas combined.

3.6.2.1 Strengths and Limitations of Dietary Assessment Methods and Results

The EPIC-Norfolk FFQ is known to significantly overestimate energy and fibre intake, as well as many macro- and micronutrients, including fat, protein, carbohydrate, potassium, calcium and carotene when compared with weighed records (Bingham 1997). This knowledge means that interpretation of the FFQ data must be done with caution. In those patients with three complete FFQs, the mean energy and protein intake falls by 90.3 kcal and 8.4 g respectively during the study (Table 3-15). The mean weight loss over this period for these patients was 5.4 kg. To get a sense of the accuracy of the FFQ, these variables can be assessed together. Assuming energy intake was reduced by 90.3 kcal/day for 12 months, this would mean a total reduction of 32,960 kcal during this time. It is known that 1 kg weight loss (assuming only fat is lost, which is unlikely) requires a reduction in energy intake of 7,700 kcal (Hall 2008). Therefore, to lose 5.4 kg, a total reduction of 41,580 kcal in 12 months would be needed. Although this is an over-

simplified approach, and weight loss caused by an increase in energy expenditure has not been considered, these calculations (difference of 8,630 kcal over 12 months) suggest that the estimated *'energy in'* using the FFQ is only slightly higher (23.6 kcal/day) than the *'energy out'* as indicated by weight loss. This indicates that the dietary adequacy picture described in Table 3-16 may be reasonably precise.

Given the many inherent limitations associated with the food frequency approach, a more accurate dietary assessment approach may have yielded more robust dietary intake data. Weighed food diaries have been advocated as the most accurate method of assessing dietary intake but given their labourious nature, they were not felt to be a practical option in this OG cancer cohort. The 7-day food diary method is considered the next best approach as it has been found to correlate closely and approach the reliability of weighed food diaries (Bingham et al. 1994; Bingham et al. 1995). This approach is feasible within a clinical environment and was strongly considered for use in this thesis. Its advantages are manifold and include precision of portion sizes and no reliance on individual memory and recall. Apart from the 16-day weighed record method, it has the next highest correlation coefficients when compared with biomarkers (urinary nitrogen and potassium, serum vitamin C and carotenoids), followed by the FFQ approach and finally the 24-hour recall method (Bingham 1997). Also, no biases in mean intakes of either foods or nutrients have been found for the 7-day food diary approach, highlighting its robustness.

However, the 7-day food diary method also has its limitations as described in Section 2.3. Previous use of the 7-day food diary in research at RM was reflected upon to determine the practicalities of using the tool in an OG cancer cohort. A prospective study assessing the management of bile acid malabsorption with low fat dietary interventions was undertaken at RM (Watson et al. 2014). Participants were asked to complete two 7-day food diaries, and the completion rate was poor: 79% (54/68) returned the first one; 57% (31/54) returned the second one. Although, this was a motivated group of patients who wished to change their diet so as to improve their GI function, these data suggest that the burden was too high for many. In

comparison, of the 194 FFQs due to be completed during the current study, just one diary (0.5%) was not returned and another one (0.5%) was incomplete on return, demonstrating the low respondent burden and the ability of patients to complete the questionnaire fully.

The 7-day food diary approach was also used in a randomised controlled trial undertaken at RM (The Fibre Study: unpublished, with permission from Ms Linda Wedlake). The degree of variation between three investigators was assessed and the mean difference between them for the daily energy intake was 174 kcal and 189 kcal for two study groups, which amounted to approximately 10% of their daily energy intake. This suggests that the estimates of total energy intake from patient-reported records varied amongst investigators. Additionally, there was evidence of systematic error in that one of the investigators consistently estimated higher energy intakes compared with the other two. Cost was another practical issue raised in this study. The time and monetary expenses of entering the diaries was very high: it took an average of 1.8 hours to enter a 7-day food diary, with a price of £30/diary (based on a Band 6 dietitian's rate of pay).

Also, with regard to dietary assessment, the use of EAR and RNI as a method of estimating whether individuals attained dietary adequacy deserves attention. Dietary reference values are intended for healthy people rather than those with a disease that may alter dietary needs, such as cancer. Given that healthy individuals obtain their nutrients from food sources alone, the reference values were not designed to cover ONS and EN too (Hurren & Ashwell 1996; Committee on Medical Aspects of Food Policy 1991). Also, the imprecision of the food frequency method in estimating an individual's nutrient intake, means that caution should be used in applying the dietary reference values to the assessment of individuals' diets. Even with the perfect measure of an individual's habitual diet (which is not easy to achieve), the dietary reference values can give no more than a guide to the adequacy of that individual's diet (Committee on Medical Aspects of Food Policy 1991). In addition, the period of time over which intakes need to be recorded varies from nutrient to nutrient, depending on the frequency of consumption of foods high in a particular nutrient, as well as, the body's method of storing it.

Therefore, as the dietary assessment period used in the current study was just one month, it is possible that the average daily intake of certain micronutrients was underestimated.

3.6.3 Small Intestinal Bacterial Overgrowth

This is the first research where multiple tests for SIBO were undertaken in patients before the commencement of treatment and during treatment for OG cancer. The prevalence of SIBO at baseline and its incidence during the study in the sub-group who underwent testing was high at 23.5% and 76.9% respectively. Despite the small number of patients who underwent testing for SIBO, the high number of positive results suggests that SIBO may be a common condition in patients with OG cancer, increasing following the start of treatment. Of those diagnosed with SIBO after baseline, 90% and 80% underwent chemotherapy and radiotherapy before test positivity. Also, those with SIBO had a number of known predisposing factors. However, as there were few (tested) individuals without SIBO it is not possible to infer causation. Likewise, although belching and nausea were reported by 43% and flatulence by 57% of those with SIBO, a much larger cohort would be required to determine which symptoms were predictive of SIBO.

3.6.3.1 Strengths and Limitations of SIBO Methods and Results

There are limitations with respect to the design of the SIBO component of this study. It had been intended that all patients in the current study would perform GHMBTs, but this was not possible because of patient reluctance to undergo the three-hour tests. This is likely to have caused a sampling bias, as the characteristics of the sub-group who underwent testing may not be comparable to the rest of the cohort. In future research, efforts to encourage all participants to perform the required GHMBTs should be made. As the test is simple to carry out and remote GHMBT kits are available, it would be possible for the patient to perform the test at home. This would reduce additional hospital attendances. In addition, patients should be encouraged to undergo testing by their medical team. A recent study explored researchers' and clinicians' perceptions of recruiting participants to clinical research and found that the formation of collaborative ties between researchers and clinicians aided recruitment, with the patient's clinician believed to make positive contributions to recruitment if they expressed encouragement

to patients (Newington & Metcalfe 2014). Also, to determine if there was a notable improvement in GI symptoms in those with a positive test, a symptom assessment after antibiotic treatment would have been ideal. In this way, only when a significant improvement in symptoms was objectively found, could SIBO be confirmed. Follow-up of this nature should ideally be incorporated into larger prospective studies to ensure a more robust diagnostic approach.

This is the first study that tested for SIBO before the commencement of OG cancer treatment, allowing for its incidence to be measured. The results provide evidence for a high prevalence of SIBO in OG cancer. However, the sources of false positive results associated with the GHMBT should be considered: incomplete absorption of glucose, production of gas by oropharyngeal bacteria and failure to avoid slowly absorbed carbohydrates. Malabsorption may occur if there is damage to the small bowel enterocytes (e.g. following chemotherapy or radiotherapy) or if intestinal transit time is rapid, which can be a feature in OG cancer patients. This is because there will be insufficient time for glucose to be completely absorbed in the proximal jejunum, thus it rapidly enters the large bowel leading to false positivity (Sellin & Hart 1992). Ideally, oro-caecal transit time would be measured alongside the GHMBT to determine if rapid motility was influencing the test result. A false positive result could also occur if bacterial fermentation by oropharyngeal bacteria is not minimised by patients maintaining good oral hygiene before the test (Thompson et al. 1985). However, in the current study, all but one patient with a positive test claimed to have cleaned their teeth before the test. Finally, the consumption of slowly absorbed carbohydrates in the 24 hours before the test can produce a high basal H_2 level, potentially resulting in a false positive result. Although three patients with positive tests did not adhere to the carbohydrate restrictions, none had raised basal H_2 levels, suggesting false positivity was not an issue here.

3.7 Conclusion

This prospective observational study is the first to focus on assessing GI symptoms and malnutrition in an OG cancer cohort during their first year after diagnosis. The burden of GI dysfunction is great and chronic: while there is some improvement in the number and severity of

symptoms acutely, the situation regresses, so that at one year, patients have reverted to pre-treatment symptom levels. There is some evidence to suggest that the cause of these GI problems may be related, at least in part, to the development of SIBO during the treatment pathway. This avenue certainly merits further research. This study describes a worrying situation with regard to nutritional status: the majority of patients are malnourished throughout their first year with OG cancer. However, hope lies in the finding that there is a relationship between the presence of GI symptoms and nutritional status. Future work may focus on establishing whether an intervention to manage GI symptoms may lead to improved nutritional status, even though a cause and effect relationship may never to realised.

Chapter 4

Comparison of the Malnutrition Universal Screening Tool with the Patient Generated Subjective Global Assessment in Patients with Oesophagogastric Cancer

4.1 Introduction

4.1.1 Rationale

As described in Chapter 3, at least 56% of patients with OG cancer were found to be moderately/suspected malnourished and a further 2-3% were severely malnourished at all three study visits, using PG-SGA. Given these high rates of malnutrition, it is important that there are effective strategies to screen nutritionally compromised patients with OG cancer. Patient Generated Subjective Global Assessment is often considered a gold-standard method to assess nutritional status in cancer patients (Ottery 2000). However, nutritional assessment should only be undertaken by a trained professional, is time-consuming, and cannot be undertaken in every patient in routine clinical practice. Therefore, an effective nutritional screening tool is essential in the oncology setting. It ensures that dietetic resources are appropriately targeted towards those patients who are most likely to require a full nutritional assessment and intervention.

European Society for Clinical Nutrition and Metabolism guidelines state that nutritional screening should be able to predict the clinical course based on nutritional status and whether patients could benefit from nutritional treatment (Kondrup et al. 2003). Many generic nutritional screening tools are available, but few are developed and tested in oncology. While the MSTC has been validated in an in-patient oncology population, this tool is based on a complex equation, which is not suitable for use in routine clinical practice (Kim et al. 2011). Therefore, despite the abundance of generic nutritional screening tools, there is no consensus on the optimal tool for use in the oncology setting.

The MUST was developed for multidisciplinary use and is supported by BAPEN, the Royal College of Nursing and the Registered Nursing Homes Association. It is the most commonly used screening tool in the UK (Stratton et al. 2004). This tool is recommended by ESPEN as the preferred screening tool for patients in the community, which is the setting for which it was originally developed (Kondrup, Allison, et al. 2003b). It predicts the rate of hospital admissions and the number of visits to general practitioners in the community. It has also been shown to

have predictive validity in the elderly hospitalised population, with regard to mortality, both in hospital and after discharge, and length of hospital stay (Stratton et al. 2006). The MUST (a) is associated with high reproducibility (degree of agreement between measurements from the same patient collected by different observers) among health care providers, (b) is internally consistent (general agreement between the items of the tool) and (c) has fair-good to excellent concurrent validity (correlates with a measure that has been validated including NRS, MST, MNA and SGA) in hospital in- and out-patients (Stratton et al. 2004; Elia 2003). Moreover, its practicability has also been documented (Stratton et al. 2004). Given this, and in the absence of a definitive method to screen for malnutrition in oncology, demonstrating the sensitivity and validity of MUST in the oncology setting would be a significant advance.

As outlined in Table 1-10, one well-conducted study has successfully attempted to validate MUST in the adult oncology setting against PG-SGA (Boléo-Tomé et al. 2011). This study was conducted in out-patients undergoing radiotherapy. However, these results cannot be generalised to patients with OG cancer having multimodality treatment, who may be in-patients. For instance, a surgical in-patient is likely to have more acute issues than an out-patient receiving radiotherapy and as such, the tool will perform differently. Given the widespread use of MUST around the UK, its excellent agreement with dietitians' assessment of malnutrition and its acceptability, it is timely that another validation study be undertaken in the oncology setting.

Sensitivity, specificity, and positive and negative predictive values are important features of nutritional screening tools. Sensitivity refers to the proportion of malnourished patients diagnosed by PG-SGA also found to be at risk of malnutrition by MUST (if a patient is truly malnourished using PG-SGA then it will be detected as such using MUST); a high sensitivity may give many false positives. Specificity refers to the proportion of well-nourished patients identified by PG-SGA also found to be at low risk by MUST (if a patient is truly well-nourished using PG-SGA then it will be detected as such using MUST); a high specificity may give many false negatives. Therefore an ideal nutritional screening tool will have high sensitivity (malnourished patients are detected as such) and high specificity (well-nourished patients are

detected as such) (Deeks 2001). This ensures that all nutritionally at risk patients are picked up, whilst also preventing any unnecessary use of dietetic resources.

Furthermore, the positive predictive value of a nutritional screening tool is the probability that a patient classified as at risk of malnutrition by MUST is effectively found to be malnourished by PG-SGA. The negative predictive value is the probability that a patient classified as not at risk of malnutrition by MUST is also defined as well-nourished by the reference method.

Sensitivity, specificity, and positive and negative predictive values are essential components of a nutritional screening tool in oncology, and were measured in this study.

4.1.2 Hypothesis

The MUST has an acceptable sensitivity and specificity (≥ 70% for both) in the OG oncology setting, by comparison with PG-SGA.

4.2 Objectives and Outcome

4.2.1 Study Objectives

The primary study objective was to assess the sensitivity and specificity of MUST against PG-SGA.

The secondary objectives were to:

1. Measure the positive and negative predictive values of MUST against PG-SGA
2. Assess the association of BMI and percentage weight loss (two components of MUST) with PG-SGA total score

4.2.2 Study Outcome

The primary study outcome was the sensitivity and specificity of MUST compared with PG-SGA.

4.3 Study Design, Population and Organisation

The study dietitian conducted this validation study as a sub-study of the GI and nutritional status study (Chapter 3), with the London-Riverside Research Committee ethical approval also covering the validation study (Appendix 8.9). As such, no additional written informed consent was required for the 80 patients with OG cancer previously described (Section 3.5.3). The study organisation and responsibilities defined in Section 3.3.2 also apply here. Nutritional screening with MUST and nutritional assessment with PG-SGA were undertaken at baseline in order that a patient's data only appeared once in the sensitivity and specificity analysis.

4.4 Methodology

4.4.1 Data Collection

For each patient, the same study dietitian (Ms Grace) undertook the nutritional screening (MUST) and nutritional assessment (PG-SGA) during the baseline study visit. The MUST screening was performed first, followed immediately by the PG-SGA. This was important as the availability of the PG-SGA results could influence the answering of the steps in MUST.

4.4.1.1 The Malnutrition Universal Screening Tool and Patient Generated Subjective Global Assessment

For MUST (details in Section 2.2.1, tool in Appendix 8.6), Step 1 involved the calculation of BMI using height and weight measurements. An account of the method used to measure these parameters has been described in Section 3.4.2.2 and was used to generate the patient's BMI. Step 2 of MUST was to estimate the percentage of unintentional weight loss over the previous three-six months. Weight loss, if applicable during this time, was calculated using previously recorded weight(s) on the hospital's EPR system. If there were no previous weight(s) recorded, the patient-reported change in weight was used.

Step 3 (acute disease effect) was determined by (a) checking for the presence of acute illness and (b) assessing nutritional intake over the previous five days and predicting intake for the forthcoming five days. Step 3 is not an objective measure. Although, three examples are

provided to illustrate acute illness (i.e. dysphagia, head injuries, undergoing GI surgery), there is no comprehensive list of acute illnesses accompanying the tool (Elia 2003). In an attempt to overcome this issue, the study dietitian undertook a local survey of oncology nurses, as they are the healthcare staff most likely to screen an OG cancer patient. This involved asking 25 nurses to list which conditions they thought a patient needed to fulfil to be considered *'acutely ill'*. Having compiled these lists, the study dietitian referred to them when undertaking the screening (Table 4-1). Following the completion of the three steps, the MUST score was calculated and enabled the categorisation of patients as being at low, moderate or high risk of malnutrition (Table 2-1).

Table 4-1 Conditions that oncology nurses report as *'acute illness'* as used in Step 3 of the Malnutrition Universal Screening Tool

• Gastrointestinal tract not functioning	• High temperature
• Gastrointestinal bleeding	• Infection (any type)
• Cardiac emergency	• Neutropenia
• Chest pain	• Septicaemia
• Difficulty breathing or on oxygen	• Tumour lysis syndrome
• Having hourly observations taken	• Spinal cord compression
• Unable to perform activities of daily living	• On intravenous fluids or antibiotics
• Non-responsive or semi-conscious	• Persistent nausea and vomiting
• Receiving palliative care	

A detailed description of the background and use of the PG-SGA tool has already been described in Sections 2.2.2 and 3.4.2.2. All the relevant sections of the PG-SGA were completed and the patient's nutritional status was then classified into one of three categories: well-nourished (SGA A), moderately/suspected malnourished (SGA B), or severely malnourished (SGA C).

4.4.2 Statistical Methodology

To enable comparisons, two categories of MUST and PG-SGA were created. For MUST they were (1) low risk of malnutrition and (2) medium and high risk of malnutrition. For PG-SGA they were (1) well-nourished and (2) moderately/suspected malnourished and severely malnourished.

Statistical analyses were conducted using SPSS 22.0 (IBM, USA). A contingency table was used to determine the sensitivity, specificity and the predictive values of MUST compared with PG-SGA. The 95% confidence intervals for sensitivity and specificity were calculated using the Adjusted Wald method. The cut-off points for sensitivity and specificity values were considered: excellent at 90-100%; good at 80-90%; fair at 70-80%; insufficient at 60-70% and poor at 50-60%. A sensitivity and specificity of 70% was set as a prerequisite for adequate performance of MUST (Academical Point System 2014).

As per the standard technique, sensitivity vs. one minus specificity (i.e. false positive rate) was plotted on a scatter plot to create a ROC curve and the area under the curve (AUC) was calculated to determine the performance of MUST (DeLong et al. 1988). The AUC is a reflection of how good the test is at discriminating between patients at risk and not at risk of malnutrition: the greater the area, the better the test. The MUST was considered to have outstanding discrimination if AUC was ≥ 0.9; excellent discrimination if AUC was 0.8-< 0.9; acceptable discrimination if AUC was 0.7-< 0.8; no discrimination if AUC was 0.5 (Hosmer et al. 2013). The ROC curve was used to determine the best cut-off point of MUST i.e. where the number of false positives and false negatives were minimised. This was done using the Youden index, which uses the maximum vertical distance from the point (x,y) on the diagonal line (chance line) of the ROC curve (Fluss et al. 2005).

Spearman's rank correlation was used to examine the linear trend between (a) BMI and (b) percentage weight loss in the previous three-six months and PG-SGA total score. Dancey and Reidy's categorisations aided the determination of the strength of the correlations: 1 is a perfect correlation; 0.7-0.9 is a strong correlation; 0.4-0.6 is a moderate correlation; 0.1-0.3 is a weak correlation; 0 is no correlation (Dancey & Reidy 2004).

4.5 Results

The baseline characteristics of the 80 newly diagnosed patients with OG cancer have previously been described (Section 3.5.3). The categorisation of malnutrition by PG-SGA and MUST are

presented in Table 4-2. The PG-SGA classified 31 (39%) as well-nourished and 49 (61%) as malnourished, of which 47 (59%) were moderately/suspected malnourished and 2 (2%) were severely malnourished. The MUST classified 42 (53%) as not being at risk of malnutrition and 38 (47%) as being at risk, of which 25 (31%) were high risk and 13 (16%) were medium risk.

Table 4-2 Cross-tabulation of Malnutrition Universal Screening Tool classification of 80 patients with oesophagogastric cancer against Patient Generated Subjective Global Assessment classification

n (%)		PG-SGA			
		Well nourished (SGA A)	Moderately/ suspected malnourished (SGA B)	Severely malnourished (SGA C)	Total
MUST	Low risk	23	19	0	**42 (53)**
	Medium risk	1	12	0	**13 (16)**
	High risk	7	16	2	**25 (31)**
	Total	**31 (39)**	**47 (59)**	**2 (2)**	**80 (100)**

The ability of MUST to predict PG-SGA is shown in Table 4-3. Twenty-eight per cent (n= 23) of patients were correctly classified by MUST as being well-nourished (true negatives) and 38% (n= 30) of patients were correctly classified as being malnourished (true positives). Twenty-four per cent (n= 19) of patients were misclassified as being well-nourished (false negatives) and 10% (n= 8) were misclassified as being malnourished (false positives).

MUST had a sensitivity in detecting patients at risk of malnutrition of 61% (95% CI: 47-74%) and specificity in detecting the well-nourished patients of 74% (95% CI: 57-87%). The positive predictive value of the tool was 79% (95% CI: 63-89%), while the negative predictive value was 55% (95% CI: 40-69%).

Table 4-3 Categorisation of patients according to the Malnutrition Universal Screening Tool in comparison with the Patient Generated Subjective Global Assessment, with calculation of sensitivity, specificity, positive predictive value and negative predictive value

MUST	PG-SGA (gold-standard)			
	Moderately/ suspected/severely malnourised (SGA B and C)	**Well nourished (SGA A)**	**Total n=**	
At risk of malnutrition (medium/high risk)	30 (True Positive)	8 (False Positive)	**38**	**Positive predictive value** 30/38= **79%**
Not at risk (low risk)	19 (False Negative)	23 (True Negative)	**42**	**Negative predictive value** 23/42= **55%**
Total, n=	**49**	**31**	**80**	
	Sensitivity: 30/49= **61%**	**Specificity** 23/31= **74%**		

The ROC curve is displayed in Figure 4-1. The AUC was 0.667 (95% CI: 0.545-0.789) for MUST, meaning the tool had a weak ability to discriminate between well-nourished individuals and those at risk of malnutrition (discrimination would have been acceptable if AUC was 0.7-< 0.8) (Hosmer et al. 2013). Therefore, MUST is unable to accurately predict the PG-SGA assessment category. Youden's index (Sensitivity + Specificity - 1) was used to establish the best cut-off point for malnutrition. The sensitivity and specificity of MUST was not improved when a cut-off score of 0.5 (rather than 1) was used: the values were the same at 61% and 74% respectively.

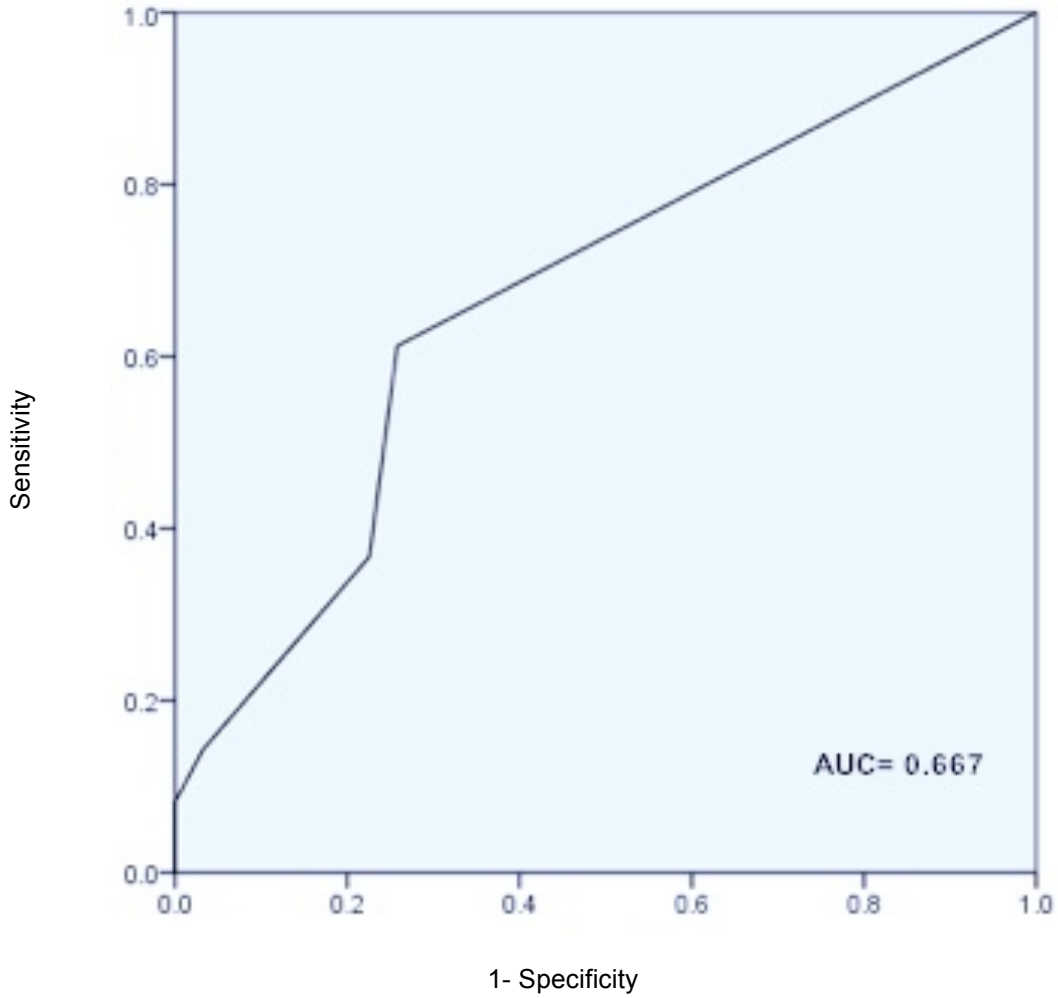
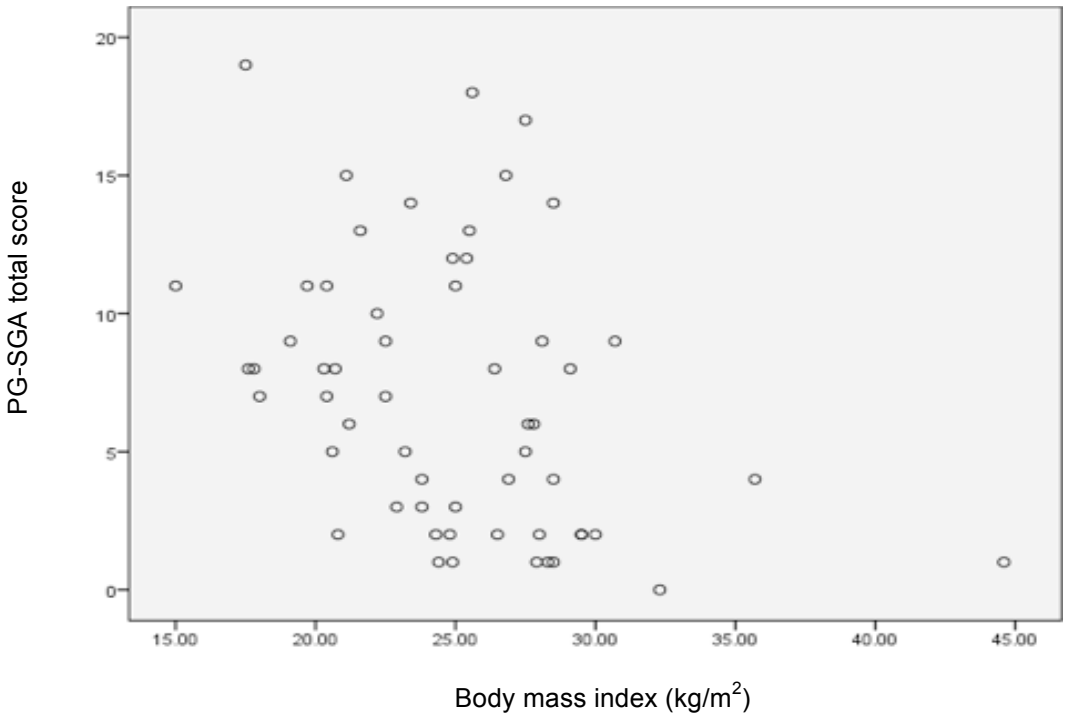


Figure 4-1 Receiver operating characteristic curve for Malnutrition Universal Screening Tool compared with Patient Generated Subjective Global Assessment

The relationships between (a) BMI and (b) percentage weight loss and PG-SGA total score were as follows: BMI and PG-SGA had a weak negative correlation, where Spearman's rank correlation coefficient was -0.259 ($p= 0.021$); percentage weight loss and PG-SGA had a moderate-strong positive correlation, where Spearman's rank correlation coefficient was +0.641 ($p< 0.001$) (Figure 4-2).

(a)



(b)

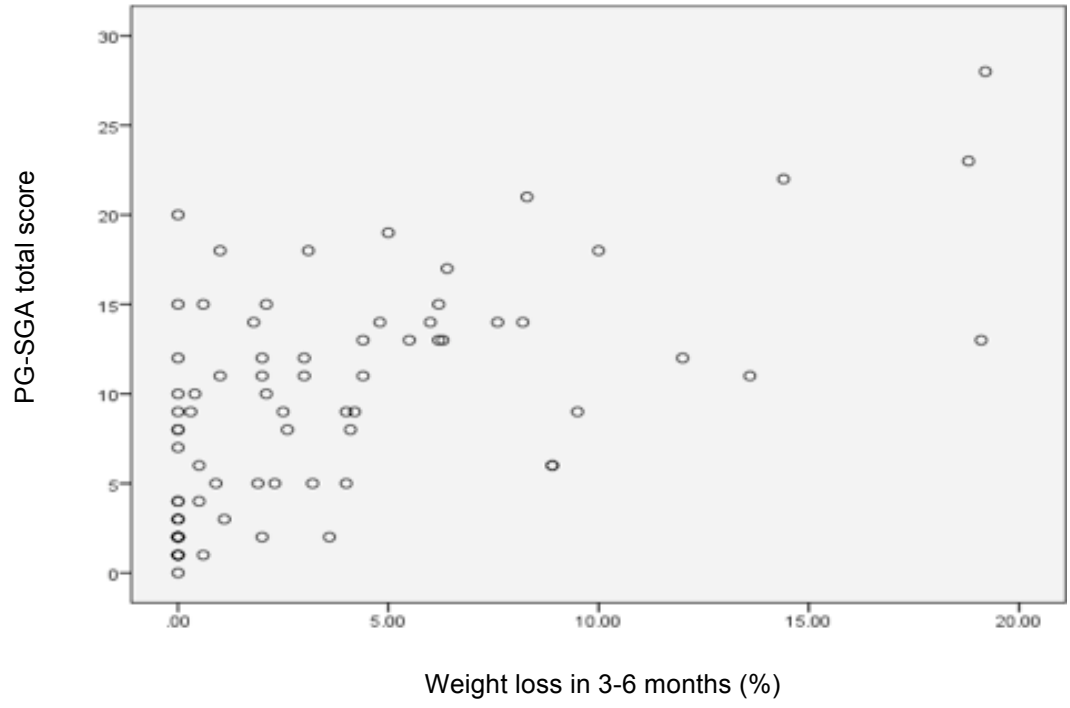


Figure 4-2 Spearman's rank correlation between Patient Generated Subjective Global Assessment total scores and (a) body mass index and (b) weight loss in previous 3-6 months: the correlation coefficients and p-values were (a) r= -0.259 (p= 0.021) and (b) r= +0.641 (p< 0.001)

4.6 Discussion

The study hypothesis was that MUST has an acceptable sensitivity and specificity in the OG oncology setting, by comparison with PG-SGA. The prerequisite sensitivity and specificity for MUST was set at 70%, the cut-off at which MUST was considered to perform adequately. Although, the specificity can be considered *'fair'* at 74%, the sensitivity is *'insufficient'* at 61% (Academical Point System 2014). Therefore, the study hypothesis cannot be accepted. Thus, although validated to be applied to all types of patient groups, based on these results, MUST does not appear to be suitable for use in the OG oncology setting.

The ideal nutritional screening tool would be 100% sensitive and 100% specific. However the ability to correctly classify all patients who are malnourished (sensitivity) takes precedence over misclassifying well-nourished patients as malnourished (specificity), because it is important that a screening tool does not let malnourished patients fall through the net and not be referred for detailed nutritional assessment and intervention, where required. Therefore a lower specificity is sometimes the compromise for high sensitivity. However, in this study, the MUST tool had a low sensitivity of 61% and a higher specificity of 74%. It failed to identify 19 patients who were malnourished according to PG-SGA.

Percentage weight loss in the previous three-six months was moderately-strongly correlated with the PG-SGA total score. This supports the findings from Boléo-Tomé's study, where three-six month weight loss (%) revealed a sensitivity and specificity of 76% and 85% respectively as compared with PG-SGA. The positive predictive value was 79% and the negative predictive value was 85% (Boléo-Tomé et al. 2011). Reported unintentional weight loss is a semi-objective measure as it relies on an individual's ability to correctly report their weight, with a systematic review of the evidence finding that adults have a tendency to underestimate their weight (Connor Gorber et al. 2007). Also, it relies on cut-offs that reflect the limits between normal and abnormal intra-individual weight changes. Despite these issues, percentage unintentional weight loss represents a valid and reliable nutritional parameter in cancer.

On the contrary, in the current study, the correlation of BMI with PG-SGA total score was weak, though the direction of the association was as expected. It is not surprising that percentage weight loss showed a stronger correlation with PG-SGA total score than BMI did, as the former is a component of the PG-SGA tool (it can contribute 5 points of a possible 49 points), while the latter is not. The results for BMI are in line with data from another study of cancer patients, where the relationship between PG-SGA total score and BMI was assessed: the correlation coefficient was -0.251 (p= 0.055) (Bauer et al. 2002). This highlights the limitation associated with using BMI as a surrogate marker of malnutrition. This is because, malnourished cancer patients may have a BMI within the healthy, overweight or obese ranges, with body fat masking loss of lean body mass (Yip et al. 2014). This is an important consideration as just 41% of men and 32% of women in England now have a BMI within normal range (Health and Social Care Information Centre 2014). In fact, the mean (SD) BMI of the current cohort was 26.7 (4.7) kg/m^2 as reported in Section 3.5.6, which places them in the overweight category. This is not surprising as previous research has shown that cancer patients often have BMIs within normal or overweight ranges e.g. the mean (SD) of 126 cancer in-patients was 26.1 (5.8) kg/m^2, and 87% of patients with metastatic cancer (n= 346) had normal or high BMIs (Shaw et al. 2014; Sarhill et al. 2003).

The prevalence of cancer-related malnutrition ranges from 26% to 80% as described in Section 1.1.4.2. The adverse effects of malnutrition in cancer are manifold: poorer responsiveness and tolerance to cancer therapies; poorer performance status; lower QoL; increased risk of infections; worse survival outcomes; and higher treatment costs (Dewys et al. 1980; van Cutsem & Arends 2005; Lis et al. 2012; Alexandre et al. 2003; Kyle et al. 2005; Andreyev et al. 1998). However, early nutritional support for those patients identified as malnourished has been shown to improve nutritional parameters, functional status as well as QoL, suggesting a global effect of nutritional intervention (Baldwin & Weekes 2011; Ravasco et al. 2003; Isenring et al. 2004; Marín Caro et al. 2007). Many studies report that nutritional counselling leads to an improvement in nutritional outcomes. A common intervention used by health-care professionals to treat malnutrition in cancer is the initiation of ONS. The use of these supplements provides a

benefit in patients who are malnourished, especially those with a BMI of < 20 kg/m^2 (Baldwin et al. 2001; Stratton & Elia 2000). A Cochrane review of nutritional supplementation showed that ONS produce a small but consistent weight gain, improvement in mortality, and shorter hospital stays (Milne et al. 2009). Increased energy and protein intake (per kg of body weight) has also been demonstrated following adherence to a daily ONS prescription in a randomised controlled trial of radiotherapy out-patients (Isenring et al. 2007).

Given the proven advantages of nutritional intervention in oncology, it is crucial that nutritional issues be addressed at the time of diagnosis and throughout the course of cancer care. To achieve this, effective nutritional screening is paramount, as it ensures that those requiring dietetic involvement are correctly identified. Nutritional screening tools should answer four questions (Kondrup, Allison, et al. 2003b): (1) What is the condition now? (2) Is the condition stable? (3) Will the condition get worse? and (4) Will the disease process accelerate nutritional deterioration?

The MUST does not adequately answer Question 3 (i.e. Will the condition get worse?). This is because the acute disease effect score of MUST (Step 3) does not consistently measure food intake. A score is only allocated to patients who are acutely ill *and* have, or are likely to have, no nutritional intake for more than five days. Interestingly, none of the 25 surveyed oncology nurses suggested that a cancer diagnosis *per se* constituted acute illness. The proportion of patients given an acute disease effect score was just 11% in the current study, which is likely to be lower than the true number of acutely ill patients. Therefore, the tool does not adequately answer Question 3 and this may have caused the high number of false negative results reported. Intriguingly, when ten community nurses undertook the same acute disease effect score survey, five believed that a cancer diagnosis *per se* was enough to warrant a score for Step 3 (assuming a reduced dietary intake too). Thus, had the community nurses criterion been use, each of the 80 patients would have been considered acutely ill and therefore, there would likely have been a lower percentage of false negatives. As such, it seems that the approach to Step 3 of MUST is a decisive factor in determining whether the tool agrees with PG-SGA.

Alternatively, the high false negative rate may be a consequence of the absence of an important element in the MUST tool i.e. there is no question related to the nutritional impact of symptoms. In the GI and nutritional status study (Chapter 3), there was a median (range) score of 3 (0-15) for the nutrition impact symptoms component of PG-SGA (for these 80 patients), indicating the contribution of symptoms to malnutrition. The assessment of symptoms would have helped to answer Question 4 (i.e. Will the disease process accelerate nutritional deterioration?).

The current study has shown that MUST lacks sensitivity and specificity in an oncology population. This confirms the findings of previous researchers. In their retrospective study, Roulston and McDermott examined the dietetic records of 52 oncology patients (primary cancer site and in-/out-patient status not reported) (2009). The MUST was compared with the clinical judgement of the oncology dietitian following a full nutritional assessment. The screening tool failed to identify 61.5% of patients considered at risk of malnutrition by the dietitian. Bauer and Capra also reported the unsuitability of MUST as a screening tool in oncology (2003). However, it should be mentioned that an early version of the tool was used in their study (the MAG screening tool) that did not yet include the acute disease effect score present in MUST. Nonetheless, their in-patient validation study (n= 65, primary cancer site not reported) of the MAG screening tool against SGA found a low sensitivity of 59% (even lower than seen here) and a specificity of 75% (a little higher than seen here). The positive predictive value was 88% and the negative predictive value 38% (negative predictive value lower than seen here). The MAG screening tool failed to identify 31% of patients who were malnourished according to SGA, one of whom was severely malnourished. Therefore, despite the adaption of the MAG screening tool to the one in use today (i.e. MUST), it appears that little has changed with regard to the tool's ability to effectively detect those patients with cancer who are truly malnourished.

The current study results, as well as those from the above two studies are not in agreement with data from a 450 patient cross-sectional study (Boléo-Tomé et al. 2011). In this study, MUST performed much better: it was successfully able to identify patients at risk of malnutrition with 80% sensitivity, 89% specificity, 87% positive predictive value and 100% negative predictive

value. It may be that the patient characteristics, treatment modalities and prognosis of those in this positive study were different from those in the three negative studies discussed, potentially affecting the performance of MUST. Roulston and McDermott did not report the patient characteristics and treatment type, while just the primary diagnoses were described by Bauer and Capra, with lymphoma (49%) and breast cancer (13%) the most prevalent. The most common diagnosis in the Boléo-Tomé study was breast cancer (21%), followed by prostate and lung cancers (19% and 16%), 44% of the group had T4 disease and all underwent radiotherapy (37% had palliative treatment). The current study specifically recruited patients with OG cancer before the commencement of radical treatment, with 60% having T3 disease. It is likely that the tool would have performed differently had the patients had T4 disease and not been treatment naïve. This may help to explain MUST's good performance in the radiation oncology cohort (Boléo-Tomé et al. 2011).

4.6.1 Strengths and Limitations

A limitation of the current validation study is the convenience sample used. However, the size of the error bands for the 95% CI for sensitivity and specificity of MUST relative to the PG-SGA were acceptable, indicating that the sample size was sufficient for this study. Also, given that only those patients with OG cancer planned for radical treatments were included in the cohort, the results cannot be generalised to the following patients: those planned for palliative therapy; those undergoing treatment or; those with cancer of another site.

A strength of this study lies in that both MUST and PG-SGA were carried out by one trained research dietitian, therefore avoiding inter-investigator variation. By undertaking the MUST before PG-SGA, the potential observer bias caused by knowing the PG-SGA category was avoided. However, therein also lies a study weakness. A dietitian completed the nutritional screening rather than a non-expert in nutrition (e.g. nurse or healthcare assistant), as would be expected. While the tool has been documented to have a high degree of reliability (low inter-observer variation), with κ= 0.88-1.00 (for nurse vs. healthcare assistant, nurse vs. student nurse, doctor vs. nurse and doctor vs. doctor), the agreement between a dietitian and a nurse or

healthcare assistant has not been studied (Elia 2000). Moreover, reliability was not measured in the current study, which is another limiting factor. As it was not measured here, one can only speculate as to whether an acceptable level of inter-observer agreement would have been achieved for a dietitian vs. a nurse/healthcare assistant. Given the nutrition knowledge of the dietitian, it is likely that MUST was completed with greater accuracy than might be expected if it were completed by a nurse/healthcare assistant. As such, the unsatisfactory results shown here may actually be an overestimate of the real sensitivity and specificity in routine clinical practice.

4.7 Conclusion

In conclusion, nutritional screening with a suitable tool should identify patients who are at the greatest risk of malnutrition. As such, dietary advice and appropriate nutritional support can be provided to those who need it. There is not yet sufficient evidence to deem one nutritional screening tool a gold-standard in the oncology setting. In this study, MUST had weak ability to discriminate between well-nourished individuals and those at risk of malnutrition. This provides evidence to suggest that MUST is unlikely to be an appropriate tool for use in OG cancer. Further validation work is required to establish if one tool is highly sensitive and specific in OG cancer as well as across all oncology groups.

Chapter 5

Exploring the Potential of Metabolomics Technology in Small Intestinal Bacterial Overgrowth Diagnosis

5.1 Introduction

5.1.1 Rationale

Small intestinal bacterial overgrowth has been recognised as a clinical entity for decades. There is some evidence to suggest that oncological treatments are predisposing factors for SIBO: pelvic radiotherapy has received attention in this regard (Husebye et al. 1994; Wedlake et al. 2008; Swan 1974), as has surgery, particularly upper-GI surgery (Brägelmann et al. 1997; Bjorneklett et al. 1983; Lock et al. 1995; Paik et al. 2011), but research is limited for chemotherapy. Also, as reported in Chapter 3, the prevalence of SIBO in OG cancer at the point of diagnosis was 23.5% (4/17), and its incidence during the first year after diagnosis was 76.9% (10/13). Of the 14 patients with SIBO, 71.4% reported flatulence and loose stools, while 64.3% and 50% reported belching and hard stools respectively. In addition, 11 (78.6%) patients were malnourished. These results and clinical experience strongly suggest that SIBO may be common in oncology patients. However, large prospective studies focused on determining its prevalence in a mixed cohort of cancer patients are lacking.

Although current testing techniques for SIBO aid with its diagnosis, they are cumbersome, insensitive and/or non-specific. A systematic review of diagnostic tests for SIBO identified neither a consistent definition nor an adequately validated test for it (Khoshini et al. 2008). Thus, the management of the condition remains difficult. Therefore, given the high prevalence of SIBO in OG cancer, the symptom burden it exerts, and the potential nutritional sequelae (Chapter 3), a test that detects the presence/absence of SIBO in patients with cancer would be a significant clinical advance.

Nuclear magnetic resonance-based metabolomics is becoming a useful tool in the study of biofluids and has the potential to contribute to the diagnosis of SIBO. Metabolomics is the comprehensive assessment of metabolites within a biological sample and attempts to systematically identify and quantify these small molecules. By identifying biomarkers, it could

lead to a simple, accurate, sensitive and specific objective test for SIBO. This would have major implications for the detection, and therefore, clinical management of this troublesome condition. At RM, there is an excellent opportunity to measure the prevalence of SIBO in oncology, describe the range of GI symptoms that it causes, as well as any biochemistry or haematological abnormalities associated with it. Additionally, the use of metabolomics technology can be trialled in this setting. It is hoped that such research will lead to an improved understanding of SIBO, more effective identification of it and ultimately, better management of the patients affected by it.

5.1.2 Hypothesis

In patients previously or currently being treated for cancer, qualitative and quantitative analyses of metabolites in urine will indicate the presence or absence of SIBO.

5.2 Study Objectives and Outcomes

5.2.1 Study Objectives

The primary study objective was to establish whether ^{1}H NMR technology allows the identification of metabolites indicative of SIBO in patients with cancer.

The secondary objectives were to:

- Describe the baseline characteristics of patients with cancer under investigation for SIBO and to compare the characteristics of the SIBO categories (Definite SIBO, Possible SIBO, No SIBO and Excluded)
- Report the prevalence and severity of GI symptoms and stool type in patients with cancer and compare these variables for the four SIBO categories in order to investigate whether specific symptoms are characteristic of SIBO
- Report the prevalence of positive and negative GHMBTs and values for H_2 and CH_4 in patients with cancer and compare results for the four SIBO categories to determine whether specific breath profiles are characteristic of SIBO

- Report the organisms grown following jejunal aspiration in patients with cancer and compare results for the four SIBO categories so as to explore whether specific organisms are characteristic of SIBO
- Describe baseline biochemistry and haematological levels in patients with cancer and compare results for the four SIBO categories to investigate whether specific variables are characteristic of SIBO
- Report the outcomes of further clinical investigations for the four SIBO categories to determine whether confounding factors were involved in SIBO diagnosis

5.2.2 Study Outcomes

The primary outcomes include:

- Four SIBO categories: Definite SIBO, Possible SIBO, No SIBO and Excluded
- Metabolite levels in baseline urine samples of patients with Definite SIBO and No SIBO

Secondary outcomes include:

- Baseline characteristics
- At baseline and follow-up (following treatment of SIBO): GSRS scores for 26 individual symptoms (0-3), GSRS total scores (0-78) and stool type when *'at best'* and *'at worst'*
- Values for H_2 and CH_4 from the GHMBT and overall test result
- Microbiology results following jejunal aspiration: bacterial strains grown and microbial growth category
- Baseline biochemistry and haematological levels
- Other GI-related conditions diagnosed after the baseline measurement

5.3 Study Design, Population and Organisation

5.3.1 Study Design and Population

This was a prospective observational case-control study conducted at RM. Patients were recruited from four specialist gastroenterology out-patient clinics. The patients included in the study were those with a historical diagnosis of cancer of any location who were either having

ongoing oncological treatment or who had previously completed treatment. There was no cut-off for how long ago this treatment was received. Treatment may have been provided for curative or palliative intent, and have been single- or multimodality in nature. Patients attended these clinics for the management of new onset GI symptoms, thought to be related to their cancer treatment. Following the baseline study visit, there was one follow-up visit to assess the impact of any antibiotic treatment on symptoms.

The study's inclusion criteria were as follows:

- Previous cancer diagnosis (any site)
- Ability to give written informed consent to participate
- Age ≥ 18 years
- Referral to the specialist gastroenterology clinic
- Suspected of having SIBO, based upon GI symptoms and clinical suspicion and investigated for such at RM

The study's exclusion criteria were as follows:

- Did not have a previous cancer diagnosis
- Inability or unwillingness to give informed consent
- Had already undergone testing for SIBO
- Had already been treated for SIBO
- Incapacity to comply with the demands of the study
- Inability to adequately understand verbal or written information given in English

5.3.2 Study Organisation and Responsibilities

The study (CCR 3736) was granted ethical approval by the National Research Ethics Service Committee (NHS London Central) on 19th December 2011 (Appendix 8.14). The RM Committee for Clinical Research authorised the study on 17th February 2012. It was approved as a single-centre study, with recruitment taking place at the hospital's London and Sutton sites. The RM Charitable Trust funded the study.

As with the GI and nutritional status study, accountability for this study rested with the Principal and Chief Investigator, Dr Jervoise Andreyev, while Dr Clare Shaw, Prof Kevin Whelan and Ms Eva Grace were co-investigators. All individuals were involved in the study design, while study progress and the daily organisation of the study was the responsibility of Ms Grace. The study was conducted in accordance with the ethical requirements of the Declaration of Helsinki (1996) and good clinical practice.

Mr Amyn Lalji was the study's database manager, with responsibilities of data protection and preservation, as well as data entry into the study database in accordance with the requirements of the Data Protection Act (1998) and with RM data protection and confidentiality arrangements. All data were treated as strictly confidential and held in a secure location. The statisticians from the Research Data Management and Statistics Unit of RM who assisted with statistical work were as follows: Ms Karen Thomas and Mr Kjell Pennert at the study design phase, Mr Pennert, Ms Clare Peckitt and Mr Kabir Mohammed at the database development, data extraction and analysis phases.

Ms Grace undertook sample processing, metabolite extraction and ^{1}H NMR analysis in the laboratories of Prof Kevin Whelan (KCL) and Dr Lindsay Edwards, Lecturer in Physiology at the Centre of Human and Aerospace Physiological Sciences, KCL. Dr Edwards and Dr Andrew Atkinson (NMR Facility Manager, Centre for Biomolecular Spectroscopy and Randall Division of Cell and Molecular Biophysics, KCL) provided support with the ^{1}H NMR spectroscopy data analysis. Following analysis, all usable data was transferred to RM as per the trust's guidelines on data transfer.

The study was discussed in the monthly research meetings as described previously. Recruitment trends, ethical issues, withdrawals and any other matters arising were discussed in this forum.

5.4 Methodology

5.4.1 Clinical Methodology

5.4.1.1 Screening, Inviting and Consenting

Each patient who attended one of the specialist gastroenterology clinics for investigation and management of GI symptoms associated with cancer or its treatment was managed with a peer-reviewed investigation and treatment algorithm (Andreyev et al. 2014). This algorithm has been shown to be highly effective in a randomised controlled trial of patients with new-onset GI symptoms persisting six months after pelvic radiotherapy (Andreyev et al. 2013). The algorithm assists clinicians to identify the cause of troublesome GI symptoms, with potential causes including: SIBO, bile acid malabsorption, exocrine pancreatic insufficiency, lactose or other disaccharide intolerance and pelvic floor dysfunction. According to this algorithm, if a patient presents to the clinician with one or more of the following GI symptoms, they are referred to the RM Endoscopy Unit for SIBO testing: bloating, flatulence, abdominal grumbling, nausea, vomiting, diarrhoea, steatorrhoea, nocturnal defaecation and constipation.

When a patient was suspected of having SIBO and had agreed to undergo investigation, they were screened against the study's inclusion/exclusion criteria and those who were eligible were invited to participate. Eligible patients were given a Patient Information Sheet by the consultant gastroenterologist, nurse consultant or study dietitian (Appendix 8.15) and informed that the study was voluntary, to prevent coercion. With permission, the study dietitian contacted the patient by telephone (or email if favoured) to determine if the information sheet had been read and if participation was considered. Any questions or queries were answered and contact details were provided.

It was important that at least 24 hours had passed before an interested patient was consented into the study. This ensured that the patient had sufficient time to fully consider all aspects of the research. If the patient agreed to participate, the baseline study visit took place at the Endoscopy Unit at the London site of RM to coincide with the appointment for SIBO testing. This avoided any additional visits to the hospital, which was important as patients had ongoing

GI symptoms, often debilitating in nature. Each patient signed a study consent form before study data were collected (Appendix 8.16). After the consent process, patients were registered into the study, but were free to withdraw at any point, without needing to provide a reason and without it affecting their management.

Each patient completed a follow-up study visit, which occurred when the patient returned to the specialist gastroenterology clinic for their routine follow-up out-patient appointment, thus avoiding unnecessary hospital visits. The modified GSRS tool captures symptom burden for the previous two-week period (Section 5.4.1.3). As the diagnosis of SIBO is largely based on the change in reported GI symptoms after treatment with antibiotics (Section 5.4.1.8), patients who received this treatment were assessed at least two weeks after the completion of the last course(s) of antibiotics. However, the actual time between baseline and follow-up study visit varied and was dependent on whether:

- The patient cancelled, postponed or did not attend their out-patient clinic appointment
- The patient received antibiotic treatment for SIBO
- There was a delay in treating the patient for SIBO
- The patient received more than one course of antibiotics

5.4.1.2 Data Collection and Entry

Once enrolled, the study dietitian collected data on a case report form (paper copy) at the two study visits. The data included on the case report form are described in Table 5-1. Details of the modified GSRS, the GHMBT, the endoscopic aspiration of jejunal fluid, sample handling and storage and the antibiotic treatment for SIBO are discussed in Sections 5.4.1.3, 5.4.1.4, 5.4.1.5, 5.4.1.6 and 5.4.1.7 respectively. The data from the case report forms was entered into a secure RM study database in a timely manner. Checks on 10% of all entered data were performed by the study dietitian before statistical analysis was permitted.

Table 5-1 CCR 3736: data collected at the two study visits

Data collected	Study visit		Where collected from
	Baseline	Follow-up	
Demographic and clinical information			
Baseline demographics	✓	✗	EPR, patient
Tumour site and histological stage	✓	✗	EPR
Presence of clinical conditions that may predispose to SIBO	✓	✗	EPR, patient
Length of time since new onset gastrointestinal symptoms	✓	✗	Patient
Biochemistry and haematology	✓	✗	EPR
Current medications that may predispose to SIBO	✓	✓	EPR, patient
New gastrointestinal diagnoses made since baseline	✗	✓	EPR
Nutritional information			
Body mass index	✓	✓	Dietitian measured height and weight
Presence of Ileostomy/colostomy	✓	✓	Patient
Treatment information			
Oncology treatment start and completion dates	✓	✗	EPR, patient
Oncological treatment received	✓	✗	EPR, patient
Antibiotic treatment for SIBO	✗	✓	EPR, patient
Questionnaire			
Modified GSRS	✓	✓	Patient
Tests for small intestinal bacterial overgrowth			
GHMBT	✓	✗	Dietitian
Endoscopic aspiration and quantification of jejunal fluid	✓	✗	Gastroenterologist and microbiologist
Samples for hydrogen nuclear magnetic resonance			
Jejunal fluid	✓	✗	Dietitian collected
Urine	✓	✓	Patient collected
Stool	✓	✓	Patient collected
Abbreviations: EPR, electronic patient record; GHMBT, glucose hydrogen methane breath test; GSRS, Gastrointestinal Symptom Rating Scale; SIBO, small intestinal bacterial overgrowth			

5.4.1.3 Modified Gastrointestinal Symptom Rating Scale

The modified GSRS was used to measure the prevalence and severity of 26 upper- and lower-GI symptoms experienced over the preceding two weeks (Section 2.1.1, Appendix 8.5). An extra four symptoms were added compared with the tool used in Chapter 3, as they were believed to be relevant to patients with SIBO. The tool will be referred to as *GSRS* for the remainder of this chapter.

5.4.1.4 Glucose Hydrogen Methane Breath Testing

For those patients willing and eligible to undertake the GHMBT, the equipment and substrate (Section 2.4.1.1), the preparation for the test (Section 2.4.1.2, Appendix 8.8), and the test protocol (Section 2.4.1.3) have previously been described.

5.4.1.5 Endoscopic Aspiration and Culture Technique

An OGD was also performed as per the investigation and treatment algorithm. This is a direct method of SIBO detection. During the procedure, jejunal fluid was aspirated for analysis. A detailed description of the test protocol for endoscopic aspiration and microbiological quantification (by culture) of the jejunal fluid has been discussed (Section 2.4.2). The microbiological cut-off values needed for test interpretation are shown in Table 2-5.

5.4.1.6 Sample Handling and Storage

When a patient agreed to participate in the study, a sample collection kit was mailed to them so that baseline stool and urine samples could be collected and delivered at the baseline study visit. The kit included the following items:

- Written instructions on the use of the kit (Appendix 8.17)
- Two 50 ml sealed screw-top, labeled collection pots (Sarstedt, UK) for storing the urine and stool samples
- Two small sealable, labeled plastic bags for holding the collection pots
- A cardboard tray for collecting the stool sample
- A wooden tongue depressor for transferring the stool from the tray to the collection pot
- Two pairs of soft vinyl examination gloves for wearing during sample collection
- A sealable, labeled opaque carrier bag for transporting the samples to RM

Patients were advised to collect the samples less than 24 hours before the baseline study visit, but ideally as close to the time of the visit as possible. When the samples were delivered to the study dietitian, they were temporarily stored in a dedicated study refrigerator, which was temperature checked daily to ensure that it was maintained between 0 and 4°C. During the

OGD, 10 ml of the jejunal aspirate sample was removed from the sterile Pennine trap before it was sealed and was transferred to a 20 ml universal container (Sarstedt, UK) and refrigerated until freezing.

Aliquots of stool, urine and jejunal aspirate samples were taken from the refrigerator and frozen at -20°C within 36 hours of having been passed/collected. The stool samples were frozen in 15 ml screw top tubes (Sarstedt, UK), and urine and jejunal aspirate samples were frozen in 5 ml cryogenic vials (Fisherbrand, UK).

At the end of the baseline study visit, each patient was provided with another sample collection kit (for urine and stool) for use before the follow-up study visit. The same collection guidelines from baseline applied at follow-up.

5.4.1.7 Antibiotic Treatment

The consultant gastroenterologist or nurse consultant commenced a regimen of antibiotic treatment in those patients with a positive GHMBT and/or aspirate culture. In situations where the patient had a positive GHMBT, the clinician chose the first, and sometimes the second line antibiotic based on whether the test was positive for H_2, CH_4 or both gases. In addition, the sensitivities of the isolates provided by the microbiology department informed the treatment decision. If both tests proved negative, the clinician may still have treated the patient with antibiotics, especially if symptoms indicated a nocturnal need to defaecate and there was still no identified cause for the ongoing GI symptoms. The choice of first and second line antibiotics was determined as per Table 5-2. Following treatment, each patient returned to the specialist gastroenterology clinic to determine the effect on GI symptoms. For patients not treated with antibiotics for SIBO, they also returned to clinic, as other interventions may have been initiated and their outcomes needed to be assessed.

Table 5-2 Standard approach to determine choice of antibiotic treatment for small intestinal bacterial overgrowth

Test results	Antibiotic choice, 1st line	Antibiotic choice, 2nd line
Glucose hydrogen methane breath test only		
H_2 positive, CH_4 positive	Ciprofloxacin	Doxycycline
CH_4 positive, H_2 negative	Doxycycline	Clarithromycin
H_2 positive, CH_4 negative	Ciprofloxacin	Doxycycline
H_2 negative, CH_4 negative … but nocturnal defaecation/ongoing symptoms with no other identified cause	Ciprofloxacin	Doxycycline
Glucose hydrogen methane breath test and jejunal aspirate		
Breath test: Any result Aspirate culture: Positive	As per sensitivities of the isolates	-
Breath test: H_2 positive, CH_4 positive Aspirate culture: Negative	Ciprofloxacin	Doxycycline
Breath test: CH_4 positive, H_2 negative Aspirate culture: Negative	Doxycycline	Clarithromycin
Breath test: H_2 positive, CH_4 negative Aspirate culture: Negative	Ciprofloxacin	Doxycycline
Breath test: Negative Aspirate culture: Negative … but nocturnal defaecation/ongoing symptoms with no other identified cause	Ciprofloxacin	Doxycycline
Abbreviation: CH_4, methane Note: If a patient reported an allergy/previous adverse reaction to a specific antibiotic, that drug was not prescribed. Antibiotic Dosages: Ciprofloxacin 1,000 mg/day for 7 days; Doxycycline, 200 mg/day on day 1 and 100 mg/day for 6 days; Clarithromycin, 1,000 mg/day for 2 days		

5.4.1.8 Diagnostic Categorising of Small Intestinal Bacterial Overgrowth

Only those patients: (a) with at least one complete test for SIBO (GHMBT or aspirate culture), (b) who attended a follow-up study visit, and (c) had completed the GSRS at both visits were considered for diagnostic categorisation. Small intestinal bacterial overgrowth status was determined by an experienced gastroenterologist (Dr Jervoise Andreyev) using a consistent approach. The diagnostic categorisation was primarily based on the patient's response to the antibiotic treatment for SIBO (i.e. change in reported GI symptoms), although the gastroenterologist was not blinded to the GHMBT and aspirate culture results. The four possible categories for SIBO status were Definite SIBO, Possible SIBO, No SIBO and Excluded and were defined using a systematic approach as described in Table 5-3.

Table 5-3 Standard approach employed by the gastroenterologist to determine small intestinal bacterial overgrowth category

Definite SIBO
Patient had an absolute unequivocal positive clinical response to the antibiotic treatment, with no other possible factors accountable for it. Plus, the patient had at least one positive test for SIBO (GHMBT or aspirate culture) and the gastroenterologist felt completely confident that he would give the patient a repeat course/courses of antibiotics should their symptoms return.
Possible SIBO
(a) Patient had an absolute unequivocal positive clinical response to the antibiotic treatment but this could possibly have been produced by factors other than antibiotics (e.g. if they had other interventions during the study period). Plus, the patient had at least one positive test for SIBO. **or** **(b)** Patient had an absolute unequivocal positive clinical response to the antibiotic treatment, with no other known factors accountable for it, but did not have a positive test for SIBO. **or** **(c)** Treatment was not given/the response to treatment was not recorded, but the patient had at least one positive test for SIBO. **or** **(d)** Patient did not have a positive clinical response to the antibiotic treatment, but had two positive tests for SIBO.
No SIBO
Patient did not have a positive clinical response to antibiotics and had at least one negative test for SIBO.
Excluded
Patient had a positive clinical response to antibiotics but factors other than antibiotics (i.e. other interventions during the study period) were strongly felt to be involved.
Abbreviations: GHMBT, glucose hydrogen methane breath test; GSRS, Gastrointestinal Symptom Rating Scale; SIBO, small intestinal bacterial overgrowth

5.4.2 Laboratory Methodology for ^{1}H NMR

The urine samples (baseline and follow-up) were analysed for metabolites using ^{1}H NMR. The laboratory methodology was adapted from that described by Beckonert and colleagues (2007). Although jejunal aspirate and stool samples were also collected as described in Section 5.4.1.6, they were not analysed for this thesis and thus will not be discussed hereafter. Due to time limitations, the pre-processing of the follow-up urine samples has not yet been performed. Therefore, for the remainder of this chapter, there will be no further reference to the follow-up urine samples.

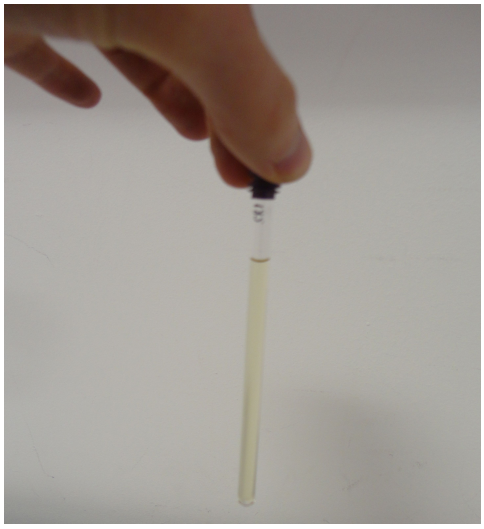
5.4.2.1 Hydrogen Nuclear Magnetic Resonance: Urine Sample Preparation

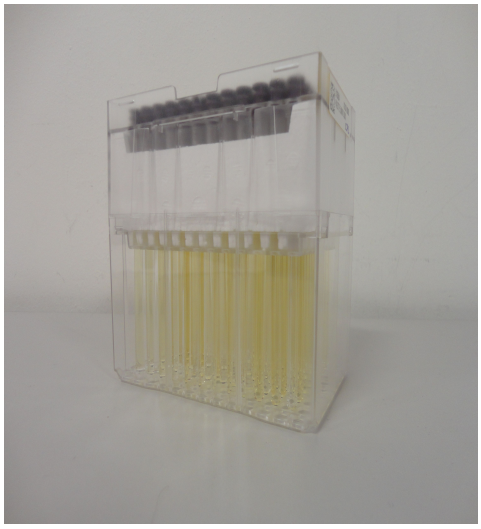
An NMR buffer solution was made up in a graduated glass bottle, to which the following were added:

- 200 ml pre-made phosphate buffered saline 0.2 M, pH 7.4 (Sigma-Aldrich, Germany)
- 0.04 g sodium azide (Sigma-Aldrich, Germany)
- 0.04 g 4, 4-dimethyl-4-silapentane-1-sulfonic acid (Sigma-Aldrich, Germany)
- 50.02 ml deuterium oxide 99.9% atom % D (Sigma-Aldrich, Germany)

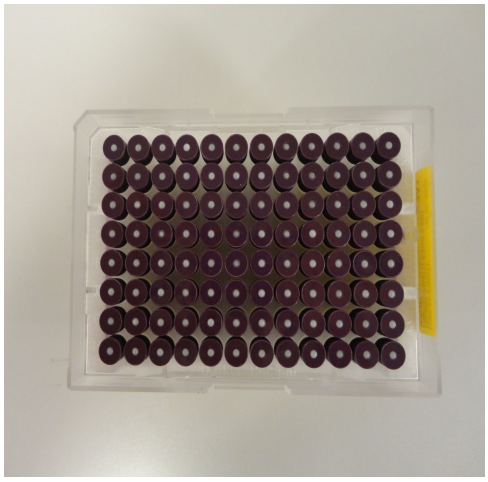
The solution was shaken thoroughly and placed on a heated stirring plate for 10 minutes. The urine samples were defrosted at room temperature, after which the samples were placed on a vortex for 30 seconds to ensure uniformity. Samples were aliquoted; 1.6 ml was aliquoted into Cryo-vials (Alpha Laboratories, UK) and re-frozen. The remaining 800 µL of each sample was mixed with 400 µL of the buffer solution in 1.5 ml microcentrifuge tubes.

The microcentrifuge tubes were then spun at 13,000 *g* for five minutes at 4°C (Forcemicro Force 1624 and Eppendorf MiniSpin Ambient microcentrifuge). Samples were frozen at -80°C until the day of analysis, when 600 µL of each sample was transferred to 5 mm NMR tubes in 96 tube racks (Bruker Biospin, Germany; Figure 5-1 a and b) using a SoftAide Pipette controller (Hamilton, Switzerland; Figure 5-1 c) and Volac 270 mm glass Pasteur pipettes (Poulten & Graf, Germany). The holes in the tube caps were then sealed using the balls provided (Bruker Biospin, Germany; Figure 5-1 d).

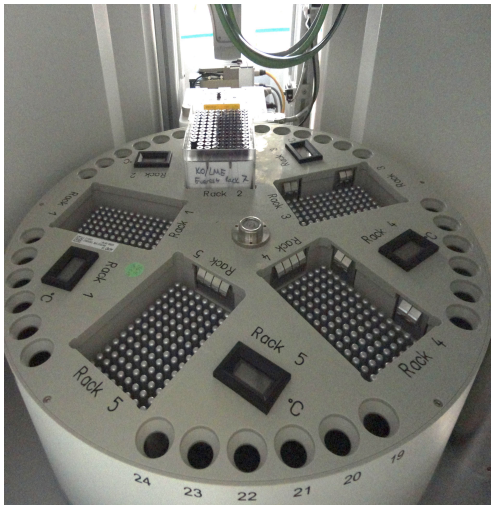

(a)


(b)

(c)


(d)

(e)



(f)

Figure 5-1 (a) 5 mm NMR tube, (b) 96 NMR tubes in rack, (c) SoftAide Pipette controller, (d) sealed NMR tube caps, (e) external and (f) internal views of 700 MHz Avance III

5.4.2.2 Hydrogen Nuclear Magnetic Resonance: Measurements (1D and 2D)

Hydrogen nuclear magnetic resonance was performed on a 700 MHz Avance III NMR Spectrometer (Bruker Biospin, Germany) that was equipped with a quadrupole resonance (QCI-P) cryoprobe and SampleJet sample changer with sample cooling (Figure 5-1 e). All experiments were performed at 298 Kelvin. Samples were moved to the SampleJet heating block to equilibrate for a minimum of one minute prior to insertion into the magnet (Figure 5-1 f). While one sample was in the magnet, the next one was moved to the heating block. Further temperature equilibration of the sample in the probe was allowed for 5 minutes.

For each sample, the probe was tuned and matched, shimming performed and the 90° pulse measured. Water suppression was achieved using the nuclear overhauser effect spectroscopy (NOESY) presaturation pulse sequence (Bruker 1D) on all samples with a spectral width of 20.52 ppm and 64,000 data points, giving an acquisition time of 2.28 seconds, a NOESY mixing time of 10 milliseconds and an inter-scan delay of 4 seconds. All spectra were recorded with an identical receiver gain.

For structure elucidation purposes, a standard 2D total correlation spectroscopy (TOCSY) experiment was recorded using a mixing time of 60 milliseconds, 224 complex points in the indirect dimension and an inter-scan delay of 5 seconds. As with 1D analysis, this was performed on a 700 MHz Avance III NMR spectrometer (Bruker Biospin, Germany) at 298 Kelvin. The 2D NMR spectroscopy is useful for increasing signal dispersion and for elucidating the connectivities between signals by plotting data in a space defined by two frequency axes rather than one. In effect, a TOCSY experiment helps to link clusters that are thought to belong to the same compound and reveal new compounds whose 1D NMR signatures could not be resolved alone.

5.4.2.3 Hydrogen Nuclear Magnetic Resonance: Data Pre-processing

Data pre-processing was performed on the baseline urine sample data using Topspin™ (Bruker Biospin, Germany) and MNova™ 9.01 (Mestrelab Research, Spain) software packages. The 1D

spectra (*'raw data'*) were pre-processed by applying a line broadening of 0.3 Hertz prior to Fourier transformation and phased using a zero order phase correction (to obtain absorption line shape). A small number of measurements gave rise to run errors. These were repeated before proceeding to the next rack of samples. Baseline correction was then performed followed by the adjustment of peaks shifts (alignment) using spectral referencing. Spectra were referenced to the deuterium oxide resonance at 0 ppm. Regions of the NMR spectrum that displayed significant resonance shifting due to variations in pH were aligned using a correlation-optimised method.

In order to reduce the data redundancy in preparation for multivariate analysis, the most widely used method is called *'binning'*. Binning is a procedure that reduces data by grouping spectral responses, with each group called a *'bin'*. The binning algorithm method used in this study was the Adaptive Intelligent Binning algorithm (De Meyer et al. 2008). With this approach, a bin edge is a point on the frequency axis, which splits the set of spectra into two new sets of partial spectra i.e. new bins. Each point in the NMR spectral range of a specific bin is evaluated as a potential (candidate) new bin edge: the best candidate bin edge is selected and the original bin is accordingly divided into two new bins. This process is repeated for each bin assuming the summed bin values of the two new bins surpass the original bin value (De Meyer et al. 2008).

Finally, samples may exhibit variable total metabolite concentrations due to instrument stability, or even the samples themselves (i.e. biological variation). For example, such inter-individual differences may occur in urine because of slight variations in pH caused by differing glomerular filtration rates. To ensure spectral intensities were directly comparable and related to concentrations, a normalisation step was needed. A probabilistic method called PQ normalisation was used, where the median quotient between all corresponding spectral data points was used as an estimate of the true dilution factor (Dieterle et al. 2006).

Following 2D analysis, the spectrum was processed in Topspin™ (Bruker Biospin, Germany) applying shifted sine-bells in both dimensions and zero-filling to the next power of two prior to Fourier transformation.

5.4.3 Statistical Methods

5.4.3.1 Sample Size Calculation

The sample size was based on a convenience sample of patients referred to the specialist gastroenterology clinics with symptoms suggestive of SIBO. Records showed that the number of eligible patients would be approximately 300 within the study recruitment period of 18 months. If 67% of these patients consented to the study (based on the research group's previous recruitment rates), a sample size of 200 was thought to be possible.

Previous data from the GI unit suggested that, of those patients tested for SIBO using the peer-reviewed algorithm, 66% were treated for SIBO, of which 50% showed a positive clinical response to treatment. Using these data and assuming a sample size of 200, this would be sufficient to fit a multivariate model using up to five variables to predict SIBO status. With a total of 66 in the Definite SIBO category, an exact 95% confidence interval for this percentage could be calculated with a lower limit of not less than 5%.

5.4.3.2 Statistical Analysis Methods

5.4.3.2.1 Clinical Outcome Statistics

Statistical analyses were conducted using SPSS (version 22.0, IBM, USA). In all statistical testing a 2-sided alpha level of 0.05 was used to assess statistical significance.

Descriptive statistics were used to describe the baseline characteristics, GI symptoms, GHMBT, microbiology and laboratory results for the whole cohort. Categorical variables were expressed as mean (SD), while continuous variables were expressed as median (range). Following the categorisation of patients (Definite SIBO, Possible SIBO, No SIBO, Excluded), analyses were performed to determine if there was any difference between the categories with respect to the

above data. For the baseline characteristics, group comparisons were performed using Kruskal-Wallis and chi-square tests.

For the individual GSRS symptoms, numerical values for each of the 26 symptoms were reported for baseline and follow-up (0= none, 1= mild, 2= moderate, 3= severe). The maximum GSRS total score was 78. Summary statistics were reported at each time point using count (percentage) of patients reporting individual symptoms, the median (range) number of symptoms and the median (range) GSRS total score**.** Kruskal-Wallis tests were used to determine any significant difference between the categories with respect to GSRS total score at baseline and follow-up and change in GSRS total score between the time points.

For the Bristol Stool Form Scale, the frequency of stool types *'at best'* and *'at worst'* were described for baseline, with groupings as follows: (a) Types 1 and 2, (b) Types 3, 4 and 5 and (c) Types 6 and 7. For patients with Definite- and No SIBO, the change in Bristol Stool Form Scale results between baseline and follow-up were reported using the cross-tabulation method.

The GHMBT results were reported as count (percentage) of positive, negative and incomplete tests for all patients at baseline. The mean (SD) highest values for H_2 and CH_4 were reported for those with positive and negative results. For the positive tests, the count (percentage) positive for H_2 alone, CH_4 alone and both gases together were reported and the proportions testing positive (for the first time) at each 20-minute time point were described. Chi-square tests were undertaken to compare the overall GHMBT results for the four diagnostic categories.

For microbiological results, the following data were reported for all patients with an OGD at baseline, using count (percentage) or median (range): aspirate appearance, microorganisms grown, strains isolated and microbiology classification (negative, intermediate or positive). Chi-square and Kruskal-Wallis tests were used to compare these data for the four SIBO categories. The results from the GHMBT were cross-tabulated against the results from the OGD, taking the

OGD to be the gold-standard: for comparison purposes the *'intermediate'* and *'positive'* OGD results were both considered positive (i.e. suggestive of SIBO).

Where biochemistry and haematological data were available at baseline, levels were expressed as median (range) for the whole cohort. To assess any differences in the four diagnostic categories Kruskal-Wallis tests were performed.

Binary logistic regression was used to compare those with Definite- and No SIBO, so as to establish any predictors of SIBO based on baseline characteristics, treatment modalities, tumour stage and selected biochemistry and haematological variables. Logistic regression was performed in a forward stepwise manner for the two patient categories, with univariate entry testing based on a score statistic of $p< 0.20$. Odds ratios were calculated to determine whether any variables were risk factors for SIBO, with the 95% CI reported and used to estimate the precision of the OR. There were considerable amounts of missing data for the univariate variables. In addition, the two patient categories had small numbers and there were many variables included in the univariate analysis. Therefore, it was not deemed appropriate to fit a multivariate model, due to the strong risk of over-fitting the model.

5.4.3.2.2 Primary Endpoint Statistical Methods: ^{1}H NMR Data Analysis and Modeling

Analysis and modeling was undertaken in RStudio™ 0.98.507 (RStudio, USA) and MATLAB™ 8.2.0.20 (MathWorks Inc., USA). The data sets described in Section 5.4.2.3 were used in dimension reducing analyses. Only the data sets of those patients considered to have Definite SIBO and No SIBO were included in the statistical procedures. Therefore, the data sets for those in the Probable SIBO and Excluded categories did not undergo data analysis or modeling.

Two approaches were taken for the untargeted analysis of baseline urine data for the two diagnostic categories (Definite SIBO, No SIBO). Firstly individual variables (bins) were compared between categories using a Welch 2-sample t-test. The false discovery rate was controlled using the standard method of Benjamini-Hochberg (false discovery rate held at 0.02

i.e. one in five would be a false discovery) (Benjamini & Hochberg 1995). Secondly, the multivariate data were reduced using PCA and PLSDA. These are both techniques used to reduce the dimension of a matrix firstly by obtaining transformed predictors and then, by fitting the model using these predictors (James et al. 2013). Principle component analysis is an unsupervised technique that involves the manipulation of a data matrix containing rows of spectra and columns of spectral descriptors. It is a bilinear decomposition method used for overviewing *'clusters'* within multivariate data and allows the expression of most of the variance within the data set. Data were visualised by plotting the PC scores i.e. the new coordinates describing the variation in the data, where each point on the scores plot represents an individual observation or sample. Partial least squares discriminatory analysis is a supervised alternative to PCA that first identifies a new set of features that are linear combinations of the original features, and then fits a linear model via least squares using these new features. Whereas PCA works to describe maximum variation between samples, PLSDA describes maximum separation between defined class samples in the data.

Principle component analysis and PLSDA indicated the bins that were the best candidates for being significant variables in the two diagnostic categories. Each bin contained multiple metabolites and the metabolite(s) causing the bin to be significant were identified. A pattern recognition, non-parametric technique called the *k*-Nearest Neighbours algorithm was used for classification using the metabolite(s). The output from this machine-learning algorithm was a class membership. A sample was classified by a majority vote of its neighbours, with the sample being assigned to the class most common among its *k*-nearest neighbours (*k* is a positive integer, typically small). The contributions of the neighbours were weighted, so that the nearer neighbours contributed more to the average than the more distant ones.

The beeswarm plot analysis method was also used for classification. This is a 2D visualisation technique where data points are plotted relative to a fixed reference axis so that no two data points overlap. It is a useful technique when the measured values of interest for each data point

wish to be seen, but also the distribution of these values. All of the data points in the plot are non-overlapping; hence, they are all visible.

The Human Metabolite Database (electronic) (Wishart et al. 2013) and Chenomx NMR Suite metabolite libraries (version 7.5 Chenomx Inc., Canada) assisted analysis of the 2D TOCSY spectra. Information from both the 1D and 2D NMR spectra confirmed assignments. Targeted profiling using Chenomx NMR Suite provided quantitative analysis.

5.4.3.3 Violations and Deviations

A patient was considered to violate the study protocol if they (a) did not have a complete GSRS at baseline or (b) had not completed at least one of the two tests for SIBO (i.e. GHMBT and aspirate culture) at baseline. Any violations resulted in the patient being excluded from the statistical analysis.

5.5 Results

5.5.1 Data Checking

There was a high accuracy of data entry into the study's database, with an error rate of < 1%, which was considered acceptable.

5.5.2 Screening

The study opened on 5th March 2012. A total of 284 patients were screened at the gastroenterology clinics, with 238 considered eligible and 46 being excluded for reasons shown in Figure 5-2. In total, 221 were invited to participate between March 2012 and May 2013. Of these patients, 21 (9.5%) declined, of which 7 (33.3%) were male and 14 (66.7%) female. The median (range) ages were 72 (69-82) years for the males and 69.5 (51-90) years for the females. The ethnic grouping of those who declined was as follows: White, 17 (81%); Other, 4 (19%); Asian/British Asian, 0 (0%); Black/Black British, 0 (0%).

5.5.3 Enrolment, Baseline Characteristics and Diagnostic Categorisations

In total, 200 patients were recruited into the study: 92 (46%) males and 108 (54%) females. The baseline characteristics of these patients are described in Table 5-4. Of the patients who consented, 179 (89.5%) were followed-up. Reasons for failed follow-up are shown in Figure 5-2.

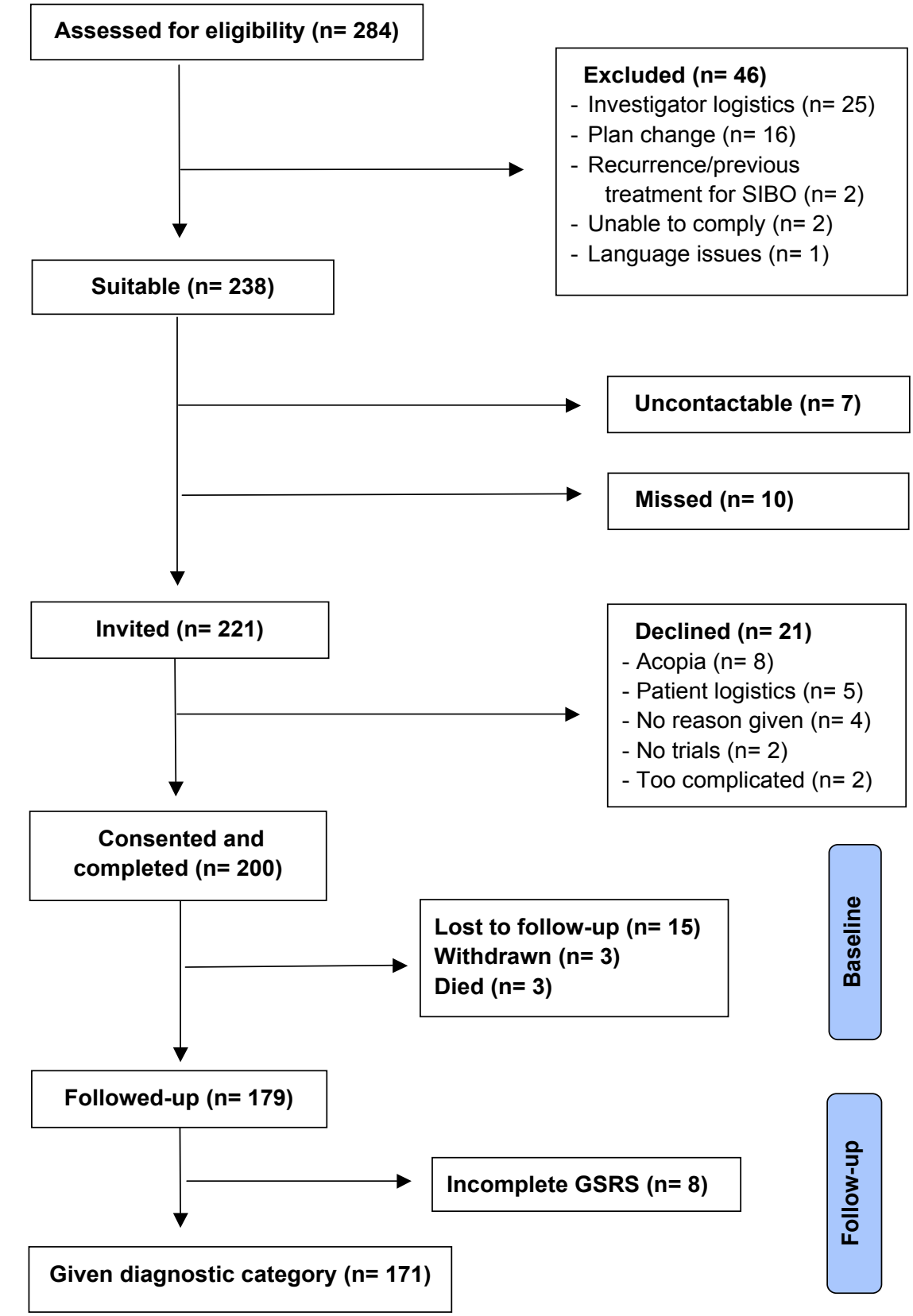


Figure 5-2 CCR 3736: screening and flow of patients through the study

Table 5-4 Baseline characteristics in recruited cohort of 200 patients with suspected small intestinal bacterial overgrowth

	n= 200
Age	***median (range) years***
Males	69 (20-89)
Females	60 (25-89)
Ethnicity	***n (%)***
White	189 (94.5)
Asian/British Asian	6 (3)
Black/Black British	3 (1.5)
Other	2 (1)
Primary tumour site	***n (%)***
Urological	65 (32.5)
Gastrointestinal	56 (28)
Gynaecological	54 (27)
Lymphoma	5 (2.5)
Haematological	6 (3)
Other	14 (7)
Histopathological tumour (T) staging	***n (%)***
0-1	26 (13)
2	48 (24)
3	46 (23)
4	18 (9)
Not applicable	15 (7.5)
Not recorded	47 (23.5)
Oncological treatment	
Treatment complete, n (%)	183 (91.5)
Months (median, range) since completion (n= 183)	35.5 (0-512)
	n (%)
Surgery	105 (52.5)
Hormone therapy	55 (27.5)
Chemotherapy	106 (53)
Radiotherapy/brachytherapy	153 (76.5)
Anthropometry (n= 195)	***mean (SD)***
Body weight	74.6 (17.6)
Body mass index	26.4 (5.7)
Predisposing factors for SIBO	***n (%)***
Gastrointestinal surgery	114 (57)
Appendectomy	33 (16.5)
Cholecystectomy	24 (12)
Hernia repair	23 (11.5)
Colonic resection	21 (10.5)
Colostomy formation	6 (3)
Small bowel resection	8 (4)
Gastrectomy	6 (3)
Splenectomy	4 (2)
Oesophagectomy	3 (1.5)
Pancreatic resection	3 (1.5)
Gastric ulcers	2 (1)
Whipple's procedure	2 (1)
Liver resection	2 (1)
Abdominal aortic aneurysm	1 (0.5)

	n= 200
Irritable bowel syndrome	26 (13)
Diabetes mellitus	19 (9.5)
Colorectal cancer	16 (8)
Lactose intolerance	6 (3)
Pancreaticobiliary disease	4 (2)
Motility disorder	2 (1)
Small bowel diverticula	2 (1)
Coeliac disease	1 (0.5)
Liver cirrhosis	1 (0.5)
Fistula	1 (0.5)
Atrophic gastritis	0 (0)
Inflammatory bowel disease	
Short bowel syndrome	
Scleroderma	
Idiopathic pseudoobstruction	
Recent medications	
Antibiotics	68 (34)
Proton pump inhibitors	59 (29.5)
Narcotics	48 (24)
Antacids	38 (19)
Antispasmodics	16 (8)
H_2-receptor antagonists	9 (4.5)

Of the 179 patients followed-up, there were 8 (4.5%) with incomplete GSRS at the follow-up visit (Figure 5-2). Therefore, 171 (95.5%) were given a diagnostic category for SIBO by the gastroenterologist, as described in 5.4.1.8. The time that had lapsed between the baseline and follow-up visit for these 171 patients was: < 4 weeks for 6 (3.5%), 4-6 weeks for 9 (5.3%), 6-8 weeks for 23 (13.4%), 8-10 weeks for 13 (7.6%), 10-12 weeks for 16 (9.4%) and > 12 weeks for 104 (60.8%). The number of patients within each diagnostic category was as follows: Definite SIBO for 38 (22.2%), Possible SIBO for 70 (40.9%), No SIBO for 45 (26.4%) and Excluded for 18 (10.5%). The 18 patients were excluded as factors other than antibiotics (i.e. other interventions during the study period) were strongly felt to be involved in the improvement of GI symptoms.

Many of the remaining results will be reported firstly for the whole cohort (n= 200) and subsequently for the sub-group with diagnostic categories for SIBO (n= 171). The baseline characteristics of the 171 categorised patients are presented in Table 5-5. The data suggests that the proportions within the four diagnostic categories were similar for all variables, except for

hormone therapy, where 40% of the patients who received hormone therapy were within the No SIBO category.

Table 5-5 Differences between the diagnostic categories (total n= 171) for baseline characteristics

	Definite SIBO, n= 38	Possible SIBO, n= 70	No SIBO, n= 45	Excluded, n= 18	p-value
Gender, Age, Ethnicity					
Gender, n (%): Male Female	18 (22.5) 20 (22)	30 (37.5) 40 (44)	25 (31.3) 20 (22)	7 (8.7) 11 (12)	0.516
Age: Median (range) years	70 (33-85)	65.5 (20-89)	67 (31-79)	59 (39-81)	0.115
Ethnicity, n (%): White Other	37 (22.5) 1 (14.3)	67 (40.9) 3 (42.9)	43 (26.2) 2 (28.5)	17 (10.4) 1 (14.3)	0.955
Primary tumour site and histopathological tumour (T) staging, n (%)					
Urological Gastrointestinal Gynaecological Lymphoma Haematological Other	10 (17.3) 13 (27.1) 11 (25) 0 (0) 1 (20) 3 (25)	21 (36.2) 22 (45.8) 17 (38.6) 4 (100) 2 (40) 4 (33.3)	22 (37.9) 9 (18.8) 8 (18.2) 0 (0) 2 (40) 4 (33.3)	5 (8.6) 4 (8.3) 8 (18.2) 0 (0) 0 (0) 1 (8.4)	0.312
Prostate cancer	10 (19.6)	17 (33.3)	20 (39.2)	4 (7.9)	0.096
0-1 2 3 4 Not applicable Not recorded	5 (23.8) 12 (27.3) 5 (13.5) 3 (18.8) 4 (30.8) 9 (22.5)	10 (47.6) 16 (36.4) 12 (32.4) 8 (50) 8 (61.5) 16 (40)	4 (19.1) 10 (22.7) 15 (40.5) 3 (18.8) 1 (7.7) 12 (30)	2 (9.5) 6 (13.6) 5 (13.5) 2 (12.4) 0 (0) 3 (7.5)	0.575
Oncological treatment, n (%)					
Surgery	24 (24.5)	39 (39.8)	21 (21.4)	14 (14.3)	0.123
Hormone therapy	11 (24.4)	12 (26.7)	18 (40)	4 (8.9)	0.038 *
Chemotherapy	19 (21.3)	42 (47.2)	19 (21.3)	9 (10.2)	0.307
Radiotherapy, brachytherapy	26 (19.8)	50 (38.2)	39 (29.8)	16 (12.2)	0.089
Predisposing factors for small intestinal bacterial overgrowth, n (%)					
One or more	29 (22.1)	51 (38.9)	36 (27.5)	15 (11.5)	0.642
Notes: Categories were compared using Kruskal-Wallis tests for age and chi-square tests for all other variables. For hormone therapy, there was a higher proportion of patients in the No SIBO category, as compared with the other three categories.					

5.5.4 Antibiotic Treatment

Of the 200 study participants, 118 (59%) received antibiotics, 76 (38%) did not receive antibiotics and it was unknown if 6 (3%) had received them for the treatment of suspected SIBO. Of the treated patients, 58 (49.2%), 53 (44.9%) and 7 (5.9) received one, two and three courses respectively. A description of the antibiotic treatment given between the baseline and follow-up time points is shown in Table 5-6.

Table 5-6 Antibiotics administered to 118 patients for the treatment of suspected small intestinal bacterial overgrowth

	1st line treatment n= 118	2nd line treatment n= 60	3rd line treatment n= 7
Antibiotic type			
Ciprofloxacin	73 (61.9)	13 (21.6)	2 (28.6)
Doxycycline	22 (18.7)	31 (51.7)	2 (28.6)
Amoxicillin/Penicillin	13 (11)	6 (10)	0 (0)
Clarithromycin	3 (2.5)	6 (10)	1 (14.2)
Metronidazole	2 (1.7)	2 (3.3)	2 (28.6)
Fluconazole	1 (0.8)	1 (1.7)	0 (0)
Other	4 (3.4)	1 (1.7)	0 (0)
Course completion as per prescription			
Completed	109 (92.4)	58 (96.7)	7 (100)
Not completed	6 (5.1)	2 (3.3)	0 (0)
Not recorded	3 (2.5)	0 (0)	0 (0)
Notes: Data presented as counts and percentages Of the 118 patients given antibiotics, 58 were given 1st line treatment only, 53 were given 1st and 2nd line treatment and 7 were given 1st, 2nd and 3rd line treatment.			

Of the 171 patients with a diagnostic category, 106 (62%) were treated with antibiotics. The antibiotic types administered to these patients are listed in Table 5-7. Overall, ciprofloxacin was the most commonly prescribed drug, followed by doxycycline. The compliance to treatment was good, with > 85% of patients in all categories completing their prescriptions.

Table 5-7 Antibiotics administered to the 106 patients with a diagnostic category

	Definite SIBO n= 38	Possible SIBO n= 70	No SIBO n= 45	Excluded n= 18
No antibiotics given	0	17	38	10
Received at least one course of antibiotics	38	53	7	8
Antibiotic type *				
Ciprofloxacin	31 (81.6)	33 (62.3)	5 (71.4)	7 (87.5)
Doxycycline	18 (47.4)	21 (39.6)	4 (57.1)	5 (62.5)
Amoxicillin/Penicillin	7 (18.4)	10 (18.9)	1 (14.2)	0
Clarithromycin	2 (5.3)	4 (7.5)	0	0
Metronidazole	1 (2.6)	4 (7.5)	0	0
Fluconazole	0	2 (3.8)	0	0
Other	2 (5.3)	2 (3.8)	0	0
All course(s) completion as per prescription				
Completed	35 (92.1)	47 (88.8)	6 (85.7)	8 (100)
Not completed	3 (7.9)	4 (7.5)	1 (14.3)	0
Not recorded	0	2 (3.7)	0	0

Notes: Data presented as counts and percentages
* Patient may have received one or more antibiotic regimens and each antibiotic listed may have been given as 1st, 2nd or 3rd line treatment. If a patient received the same antibiotic twice/three times, this was only counted once.

5.5.5 Gastrointestinal Symptoms

At baseline, the median (range) time since the onset of new GI symptoms for the whole cohort was 24 (2-372) months and the median (range) GSRS total score was 21.5 (3-56). The median (range) number of GI symptoms was 13 (3-26). The reported prevalence of the individual GI symptoms at baseline is shown in Figure 5-3. The most commonly reported symptoms (any severity rating) were flatulence (95%), faecal urgency (87%), loose stools (83%) and a negative change in the frequency of stool (77%). The symptoms that were reported least often were dysphagia, odynophagia and regurgitation (all apply to solids and fluids), with between 6% and 26% of patients reporting them.

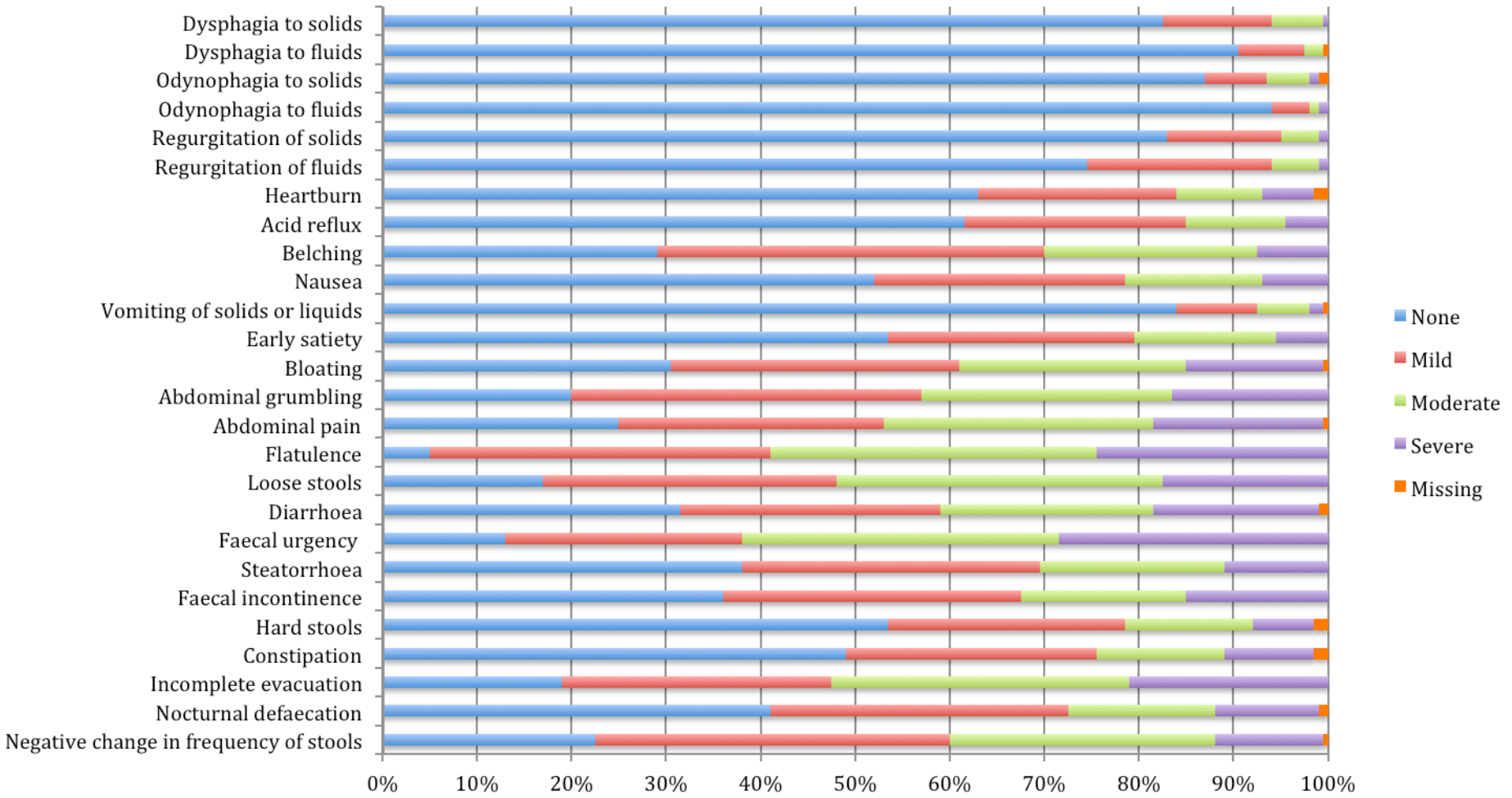


Figure 5-3 The prevalence of symptoms at baseline using the Gastrointestinal Symptom Rating Scale (n= 200)

At baseline, the median (range) GSRS total score was 22 (3-56) for the 171 categorised patients, which is the very similar to that of the whole cohort (n= 200). The reported prevalence of the individual GI symptoms at baseline for the four categories is shown in Figure 5-4 and Figure 5-5.

In those with Definite SIBO, the most commonly reported symptoms were flatulence (97%), faecal urgency (92%), loose stools (89%), abdominal grumbling (89%) and incomplete evacuation (84%). Comparing the prevalence of the 26 symptoms between the Definite- and No-SIBO, 18 of the 26 symptoms were more prevalent in those with Definite SIBO, five were more prevalent in those with No SIBO and three had the same prevalence between the categories. The symptoms with the greatest difference between the two categories were steatorrhoea (20% points higher in Definite SIBO), abdominal grumbling (20% points higher in Definite SIBO), belching (19% points higher in Definite SIBO), regurgitations of fluids (16% points higher in Definite SIBO), heartburn (12% points higher In No SIBO) and loose stools (11% points higher in Definite SIBO).

For the 171 categorised patients, median (range) number of GI symptoms was 13 (3-26) at baseline and 3 (0-11) at follow-up. Table 5-8 shows the comparison of (a) number of GI symptoms, (b) GSRS total scores and (c) change in GSRS total scores at baseline and follow-up, so as to determine any significant difference between the four SIBO categories for these variables. No significant differences were noted.

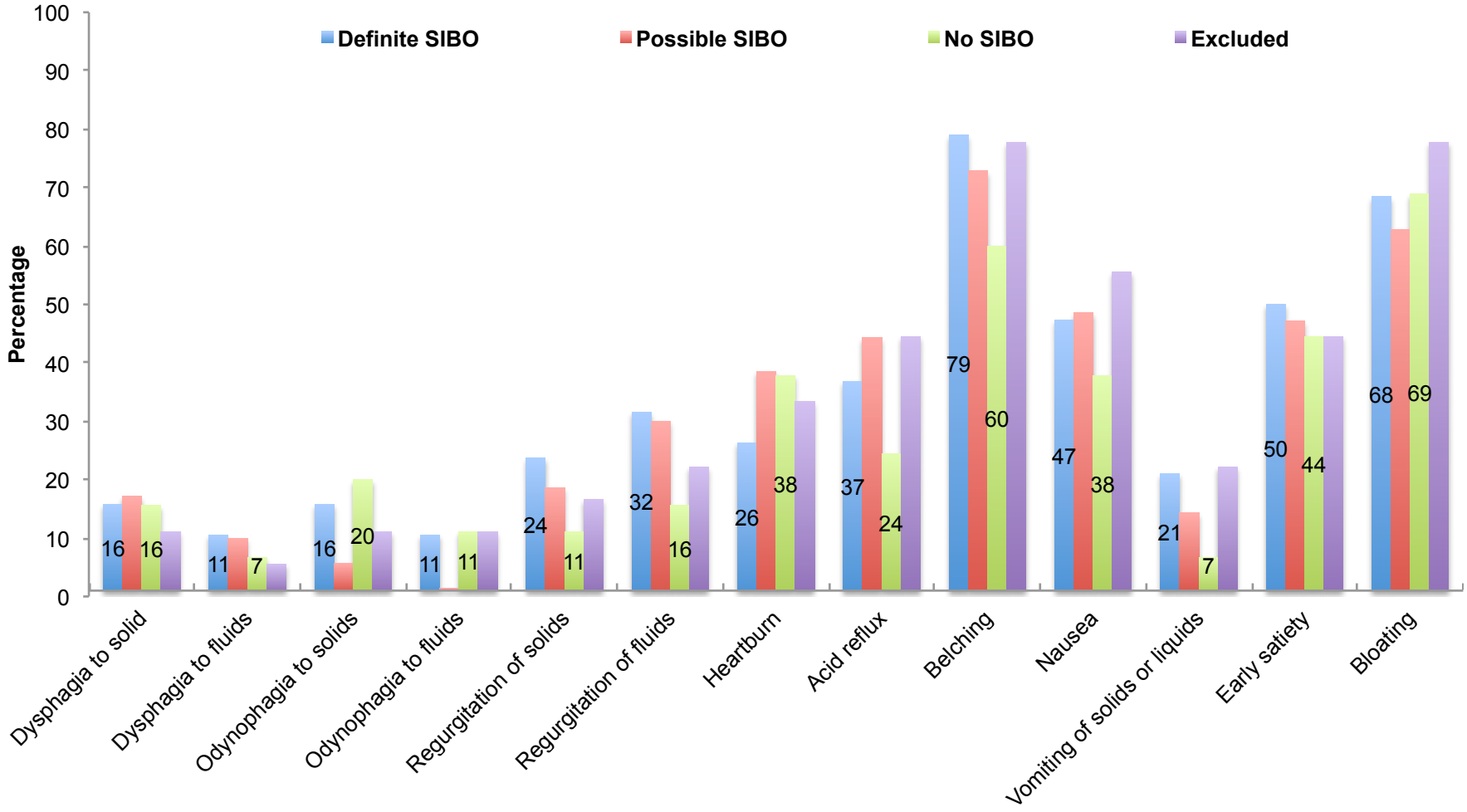


Figure 5-4 The prevalence of upper-gastrointestinal symptoms (rated as mild, moderate or severe) at baseline in the 171 categorised patients: Definite SIBO (n= 38), Possible SIBO (n= 70), No SIBO (n= 45), Excluded (n= 18)

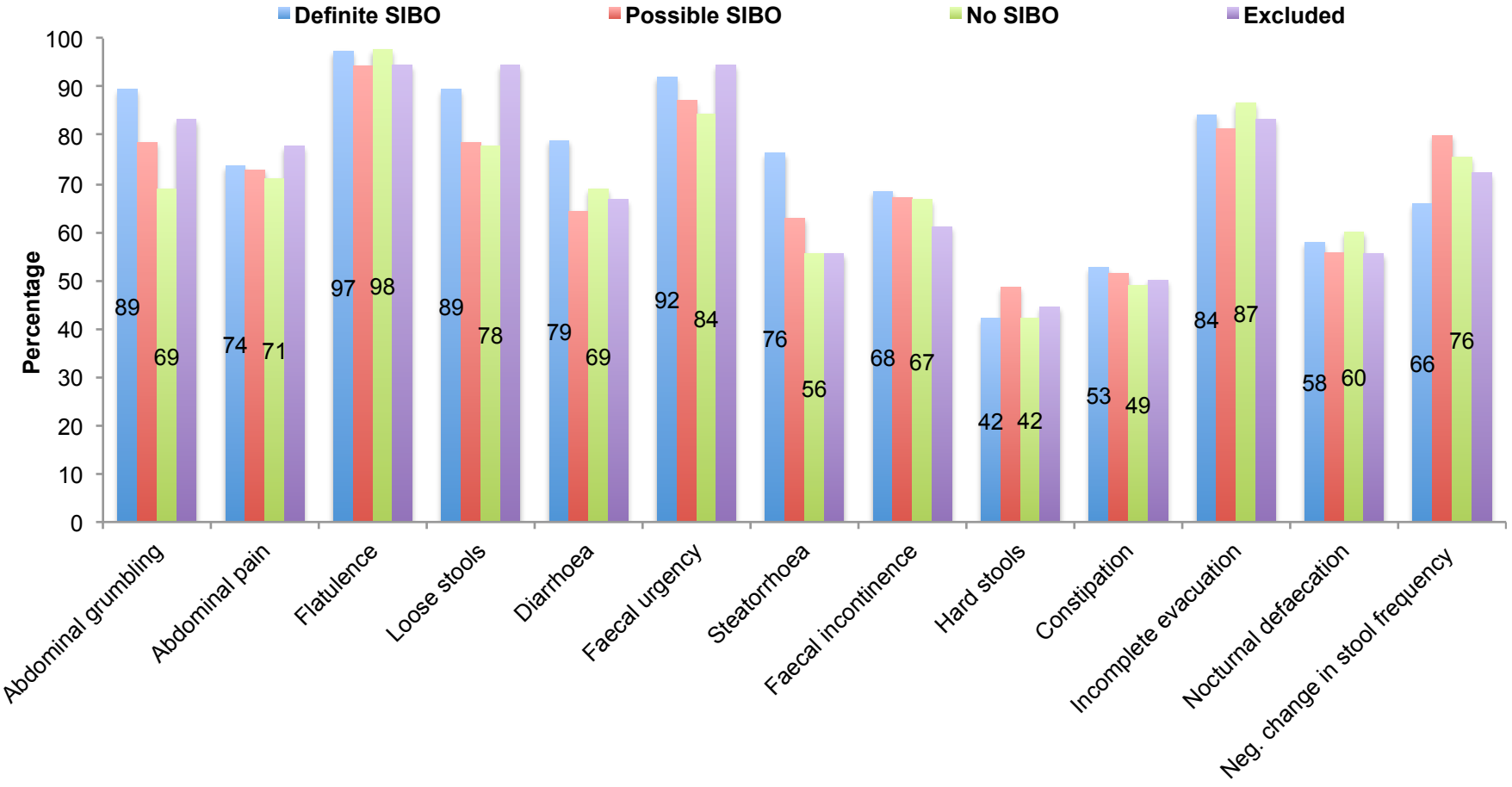


Figure 5-5 The prevalence of lower-gastrointestinal symptoms (rated as mild, moderate or severe) at baseline in the 171 categorised patients: Definite SIBO (n= 38), Possible SIBO (n= 70), No SIBO (n= 45), Excluded (n= 18)

Table 5-8 Differences between the diagnostic categories (total n= 171) for (a) number of gastrointestinal symptoms, (b) Gastrointestinal Symptom Rating Scale (GSRS) total scores and (c) change in GSRS total scores

	Definite SIBO n= 38	Possible SIBO n= 70	No SIBO n= 45	Excluded n= 18	p-value
Number of GI symptoms					
Baseline					
Median (range)	13.5 (5-26)	13 (4-24)	13 (3-20)	13 (6-24)	-
Follow-up					
Median (range)	3 (0-10)	3 (0-11)	2 (0-8)	3 (0-9)	-
GSRS total scores					
Baseline					
Median (range)	23.5 (7-52)	22 (4-56)	20 (3-44)	20 (8-50)	0.377
Follow-up					
Median (range)	19 (4-53)	16.5 (0-49)	16 (1-40)	16 (2-45)	0.916
GSRS total score change between baseline and follow-up					
Median (range)	7 (-7: 27)	5 (-22: 48)	4 (-15: 22)	3 (-15: 25)	0.259

Notes: Categories were compared using Kruskal-Wallis tests. Post-hoc tests were not conducted, as there were no significant differences between the categories.

The baseline Bristol Stool Form Scale results for the 200 patients are reported in Figure 5-6. There were 79.5% of patients who reported having Type 3, 4 or 5 *'at best'* but 72% who reported having Type 7 when *'at worst'*.

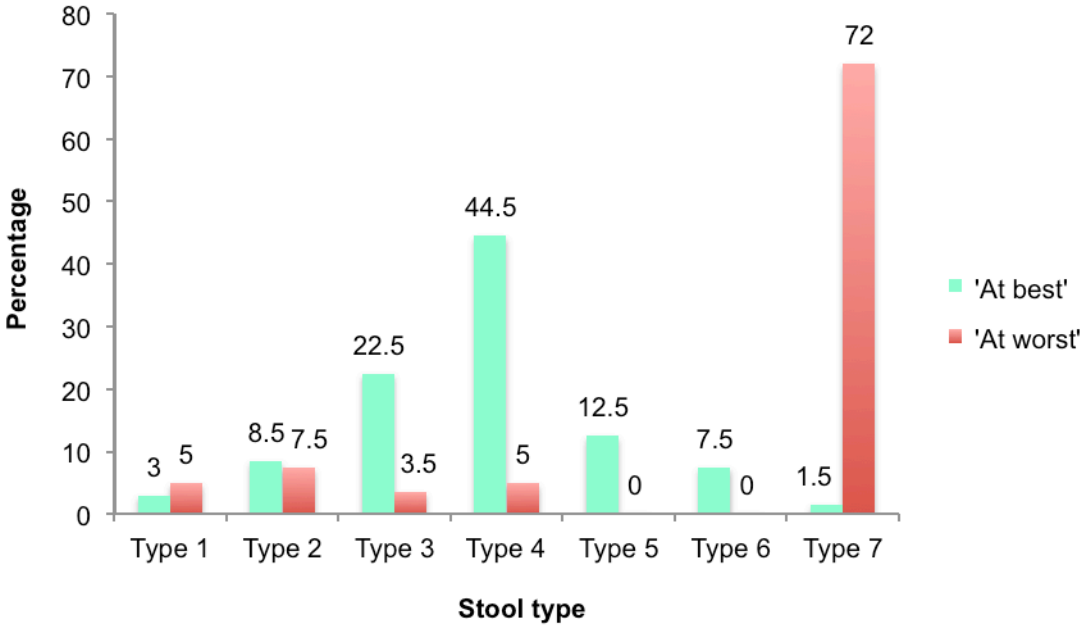


Figure 5-6 The reported prevalence of Bristol Stool Form Scale stool types *'at best'* and *'at worst'* at baseline (n= 200)

For the patients categorised as having Definite SIBO (n= 38) and No SIBO (n= 45), the change in Bristol Stool Form Scale results between baseline and follow-up are reported in Table 5-9. At baseline, 27 (72.9%) and 30 (66.7%) of those with Definite- and No SIBO respectively had Type 6 or 7 stool when *'at worst'*. Of these 27 (Definite SIBO) and 30 (No SIBO) patients, 14 (51.9%) and 10 (33.3%) no longer had Type 6 or 7 stool when *'at worst'* at follow-up.

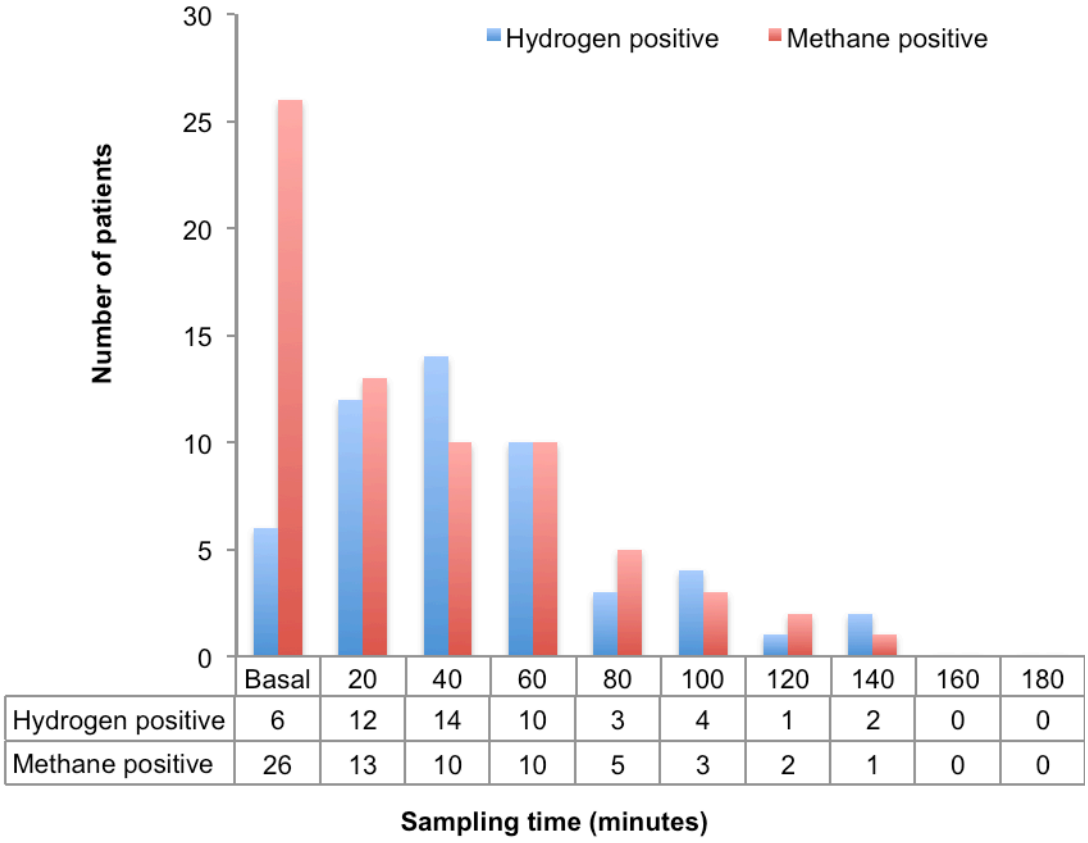
Table 5-9 Cross-tabulation of stool types *'at best'* and *'at worst'* at baseline and follow-up for those with Definite SIBO and No SIBO using the Bristol Stool Form Scale

'At best'					
Definite SIBO [a]		**Baseline**			
		Type 1, 2	**Type 3, 4, 5**	**Type 6, 7**	**Total**
Follow-up	**Type 1, 2**	0	1	0	1
	Type 3, 4, 5	3	31	2	36
	Type 6, 7	0	0	0	0
	Missing	0	1	0	1
	Total	3	33	2	38
No SIBO [b]		**Baseline**			
		Type 1, 2	**Type 3, 4, 5**	**Type 6, 7**	**Total**
Follow-up	**Type 1, 2**	1	3	0	4
	Type 3, 4, 5	4	33	3	40
	Type 6, 7	0	0	1	1
	Total	5	36	4	45
'At worst'					
Definite SIBO [c]		**Baseline**			
		Type 1, 2	**Type 3, 4, 5**	**Type 6, 7**	**Total**
Follow-up	**Type 1, 2**	3	0	4	7
	Type 3, 4, 5	1	4	10	15
	Type 6, 7	2	0	13	15
	Missing	0	0	1	1
	Total	6	4	28	38
No SIBO [d]		**Baseline**			
		Type 1, 2	**Type 3, 4, 5**	**Type 6, 7**	**Total**
Follow-up	**Type 1, 2**	8	1	2	11
	Type 3, 4, 5	2	1	8	11
	Type 6, 7	1	2	20	23
	Total	11	4	30	45

Notes: Data expressed as number of patients.
Key: Blue cell= looser stool; yellow cell= firmer stool.
[a] 31/37 (84%) no change, 3/37 (8%) looser stool, 3/37 (8%) firmer stool
[b] 35/45 (78%) no change, 4/45 (9%) looser stool, 6/45 (13%) firmer stool
[c] 20/37 (54%) no change, 3/37 (8%) looser stool, 14/37 (38%) firmer stool
[d] 2/45 (64%) no change, 5/45 (11%) looser stool, 11/45 (25%) firmer stool

5.5.6 Glucose Hydrogen Methane Breath Testing

One hundred and ninety three (96.5%) patients underwent a GHMBT. Of these, there were 191 complete tests and 2 incomplete tests. Of the 191 assessable tests, there were 79 (41.4) that proved positive and 112 (58.6) that proved negative at three hours. The mean (SD) highest H_2 and CH_4 values for those with positive results were 28.3 (27.1) and 20 (15); the mean (SD) highest H_2 and CH_4 values for those with negative tests were 4.9 (4.3) and 3.7 (3.3). Of the positive tests, there were 9 (11.4%) positive for H_2 alone, 27 (34.2%) positive for CH_4 alone and 43 (54.4%) positive for both gases. As such, there were 52 tests positive for H_2 and 70 positive for CH_4 in total. The timings of test positivity are described in Figure 5-7. Of note, no test became positive after the 140-minute sampling point and there were just 5 patients (6.3%) with a positive test at the 120- and 140 minute sampling points (one of whom was positive for both gases).



	Basal	20	40	60	80	100	120	140	160	180
Hydrogen positive	6	12	14	10	3	4	1	2	0	0
Methane positive	26	13	10	10	5	3	2	1	0	0

Figure 5-7 Timing of positivity of 79 glucose hydrogen methane breath tests, where a test can be positive for hydrogen, methane or both gases

Ten (12.7%) patients with a positive test did not adhere to the pre-test guidelines described in Section 2.4.1.2: 5 (6.3%) ate slowly absorbed carbohydrates during the previous 24 hours; 3 (3.8%) did not brush teeth/use mouthwash on the morning of the test; 1 (1.3%) took sugar-coated medication on the morning of the test; 1 (1.3%) ate food during the 12-hour fasting period and did not brush teeth/use mouthwash on the morning of the test. Of these ten patients, two had raised H_2 levels at baseline (i.e. positive test at baseline), one of which was also positive for CH_4.

Of the 171 categorised patients (Definite SIBO, Possible SIBO, No SIBO and Excluded), 169 (98.8%) undertook a GHMBT, 2 (1.2%) of which were incomplete. The counts and proportions of those with positive and negative tests are summarised in Table 5-10. Overall, a positive GHMBT can be associated with Definite- and Possible SIBO categories, rather than No SIBO and Excluded categories.

Table 5-10 Glucose hydrogen methane breath test results for the 171 patients with a diagnostic category and comparison between categories

	Definite SIBO n= 38	**Possible SIBO n= 70**	**No SIBO n= 45**	**Excluded n= 18**	**p-value**
Test result according to the H_2 and CH_4 components					
H_2 negative, CH_4 negative	10 (10.3) [a]	34 (35.1)	44 (45.4)	9 (9.2)	< 0.001
H_2 positive, CH_4 negative	2 (22.2)	7 (77.8)	0 (0)	0 (0)	
H_2 negative, CH_4 positive	11 (44)	12 (48)	1 (4)	1 (4)	
H_2 positive, CH_4 positive	15 (41.7)	17 (47.2)	0 (0)	4 (11.1)	
Incomplete	-	-	-	2	-
Missing	-	-	-	2	
Overall result					
Positive	28 (40)	36 (51.4)	1 (1.4)	5 (7.2)	< 0.001
Negative	10 (10.3)	34 (35.1)	44 (45.3)	9 (9.3)	
Incomplete or missing	-	-	-	4	-

Abbreviations; CH_4, methane; SIBO, small intestinal bacterial overgrowth.
Notes: Data expressed as counts (percentages).
[a] All 10 patients had a positive jejunal aspirate culture result.
Chi-square tests were performed to assess difference in proportions between the categories, excluding those with incomplete tests: overall, a positive GHMBT can be associated with Definite- and Possible SIBO categories, rather than No SIBO and Excluded categories.

5.5.7 Endoscopic Aspiration and Culture

Of the 200 recruited patients, 182 patients (91%) underwent an OGD with jejunal aspiration and microbiological quantification of the sample. The appearance of the aspirate on collection, the number and types of microorganism isolated during microbiological assessment and the microbiological classification of these patients is presented in Table 5-11. There were 15 (8.2%) patients considered to have a positive result based on the pre-defined microbiological cut-off levels (Table 2-5). Of the 171 categorised patients, 157 (92%) underwent endoscopic aspiration and their microbiological results are presented in Table 5-12. Overall, having isolated bacterial strains and higher bacterial counts was associated with Definite- and Possible SIBO categories, rather than No SIBO and Excluded categories.

Table 5-11 Endoscopic aspiration and microbiological results for the 182 patients who underwent an oesophago-gastroduodenoscopy with jejunal fluid aspiration

Aspirate appearance, n (%)	
Crystal clear	26 (14.3)
Cloudy	47 (25.8)
Bile stained	84 (46.2)
Not recorded	25 (13.7)
Microorganisms grown in CFU/ml, median (range)	
Aerobic Gram-positive bacillus	0 (0-1,000,000)
Aerobic Gram-negative bacillus	0 (0-1,000,000)
Anaerobic bacteria	0 (0-55,000)
Candida	0 (0-10,000)
Total	0 (0-1,055,000)
Strains isolated, n (%)	
No Yes * Coliforms Giardia Streptococcus Candida Klebsiella Other	102 (56) 80 (44) 2 (2.5) 0 (0) 45 (56.3) 8 (10) 41 (51.3) 48 (60)
Microbiology classification, n (%)	
Negative	139 (76.4)
Intermediate	28 (15.4)
Positive	15 (8.2)
***** Multiple strains may be isolated in a single aspirate	

Table 5-12 Endoscopic aspiration and microbiological results for the 157 patients with a diagnostic category who underwent an oesophago-gastroduodenoscopy with jejunal fluid aspiration and comparison between categories

	Definite SIBO n= 38	Possible SIBO n= 70	No SIBO n= 45	Excluded n= 4	p-value
Aspirate appearance, n (%): n= 135					
Crystal clear	4 (12.1)	8 (13.1)	7 (18.9)	0	
Cloudy	11 (33.3)	18 (29.5)	10 (27.0)	1 (25)	0.930
Bile stained	18 (54.6)	35 (57.4)	20 (54.1)	3 (75)	
Microorganisms grown in CFU/ml, median (range)					
Aerobic Gram-positive bacillus	0 (0-100,000)	0 (0-1,000,000)	0 (0-10,000)	5,000 (0-10,000)	< 0.001
Aerobic Gram-negative bacillus	0 (0-1,000,000)	0 (0-1,000,000)	0 (0-500,500)	55,000 (0-150,000)	< 0.001
Anaerobic bacteria	0 (0-55,000)	0 (0-10,000)	0 (0-0)	0 (0-0)	0.344
Candida	0 (0-10,000)	0 (0-10,000)	0 (0-0)	0 (0-0)	0.007
Total	12,750 (0-1,000,000)	5,864 (0-1,055,000)	0 (0-500,500)	65,000 (0-150,000)	< 0.001
Strains isolated, n (%): n= 132					
No	9 (25.7)	25 (41.7)	29 (87.9)	1 (25)	
Yes *	26 (74.3)	35 (58.3)	4 (12.1)	3 (75)	< 0.001
Coliforms	2 (5.7)	0 (0)	0 (0)	0 (0)	
Giardia	0 (0)	0 (0)	0 (0)	0 (0)	
Streptococcus	13 (37.1)	21 (35)	1 (3)	2 (50)	
Candida	3 (8.6)	2 (3.3)	0 (0)	0 (0)	
Klebsiella	14 (40)	17 (28.3)	3 (9.1)	1 (25)	
Other	18 (51.4)	21 (35)	2 (6.1)	3 (75)	
Microbiology classification, n (%)					
Negative	19 (50)	56 (80)	43 (95.5)	1 (25)	
Intermediate	14 (36.8)	9 (12.9)	2 (4.5)	1 (25)	
Positive	5 (13.2)	5 (7.1)	0 (0)	2 (50)	

Notes: ***** Multiple strains may be isolated in a single aspirate.
Chi-square tests were performed to assess differences between the categories for the aspirate appearance and strains isolated and Kruskal-Wallis tests were performed to assess differences between the categories for the microorganisms grown. Significant variables were adjusted for multiple comparisons. Post-hoc tests (pair-wise comparisons) revealed the following significant differences:

Definite SIBO vs. No SIBO: There were significantly higher levels of aerobic Gram-positive bacillus ($p= 0.001$), aerobic Gram-negative bacillus ($p< 0.001$), Candida ($p= 0.011$) and total growth ($p= 0.001$) in those with Definite SIBO.

Definite SIBO vs. Possible SIBO: There were significantly higher levels of aerobic Gram-negative bacillus ($p= 0.018$) and Candida ($p= 0.015$) in those with Definite SIBO.

Possible SIBO vs. No SIBO: There were significantly higher levels of aerobic Gram-positive bacillus ($p< 0.001$) and total growth ($p< 0.001$) in those with Possible SIBO.

Possible SIBO vs. Excluded: There were significantly higher levels of aerobic Gram-negative bacillus ($p= 0.032$) in those with Possible SIBO.

Excluded vs. No SIBO: There were significantly higher levels of aerobic Gram-negative bacillus ($p= 0.004$) in those Excluded.

Of the whole cohort, there were 153 patients who underwent a jejunal aspirate and had a complete GHMBT. The two test results are compared in Table 5-13. When the *'positive'* and *'intermediate'* aspirates were combined and taken as being indicative of SIBO (i.e. a positive result), concurrent results occurred in 58.2% of available cases, with non-concurrent results occurred in the remaining 41.8% of available cases.

Table 5-13 Cross-tabulation of glucose hydrogen methane breath test and jejunal aspiration results (n= 200)

		Glucose hydrogen methane breath test			
		Positive	**Negative**	**Missing**	**Total**
Jejunal aspirate	**Positive**	5	5	2	12
	Intermediate	13	12	1	26
	Negative	47	71	1	119
	Missing	14	24	5	43
	Total	79	112	9	200

'Missing' breath tests include those that are missing or incomplete.
'Positive and *'Intermediate'* jejunal aspirate results were both considered a positive result.
Concurrent results: 89/153 = 58.2% (green shading).
Nonconcurrent results: 64/153= 41.8% (red shading).

5.5.8 Biochemistry and Haematological Results

For all biochemistry and haematological variables, there were data available for at least some of the 200 patients at baseline, although there were no variables with data available for every patient (Table 5-14). Results for the 171 categorised patients are shown in Table 5-15. Following adjustments for multiple comparisons, there was found to be no significant differences between the laboratory variables for those with Definite SIBO and No SIBO.

Table 5-14 Available biochemistry and haematological results at baseline for the cohort of 200 patients

	Number of patients	Median (range)
General biochemistry		
Creatinine, µmol/L	195	71 (31-145)
Glucose, mmol/L	186	5.5 (3.1-9)
Urea, mmol/L	190	5.2 (1.8-9.9)
Full blood count		
Haemoglobin, g/dL	188	13.2 (10-16.7)
Platelets, $x10^9$/L	197	233 (101-461)
Red blood cells, $x10^{12}$/L	195	4.4 (3-5.6)
Liver function tests		
Alanine transaminase, µ/L	193	22 (8-91)
Alkaline phosphatase, µ/L	193	63 (29-770)
Aspartate transaminase, µ/L	9	24 (13-45)
Bilirubin (total), µmol/L	194	11.5 (0-77)
Gamma-glutamyl transpeptidase, µ/L	101	22 (1-440)
Protein levels		
Albumin, g/L	194	41 (23-49)
C-reactive protein, mg/L	182	2 (0-61)
Ferritin, ng/mL	10	87.5 (30-161)
Hormone levels		
Thyroid-stimulating hormone, µmol/L	180	1.5 (0.03-6.6)
Free thyroxine, pmol/L	179	14.2 (1.9-26.2)
Vitamin levels		
Vitamin A, µmol/L	101	1.9 (0.7-3.5)
Vitamin B_{12}, pg/mL	170	254.5 (101-998)
Vitamin D, µmol/L	111	48 (19-255)
Vitamin E, µmol/L	101	31.7 (16.9-62)
Red cell folate, ng/mL	170	476.5 (192-1,289)
Serum folate, ng/mL	168	7.3 (1.6-725)
Trace elements		
Iron, µmol/L	179	13 (3-50)
Selenium, µmol/L	93	1.1 (0.2-3.5)
Zinc, µmol/L	84	12.7 (10-29.3)
Miscellaneous		
Erythrocyte sedimentation rate, mm/hr	133	15 (1-93)
Total iron-binding capacity, µmol/L	177	58 (31-86)

Table 5-15 Biochemistry and haematological results for the 171 patients with a diagnostic category and comparison between categories

	n=	Definite SIBO n= 38	n=	Possible SIBO n= 70	n=	No SIBO n= 45	n=	Excluded n= 18	p-value
General biochemistry									
Creatinine, µmol/L	37	75 (39-143)	69	68 (31-128)	44	73.5 (31-134)	18	66.5 (48-145)	0.177
Glucose, mmol/L	35	5.7 (3.4-8.9)	64	5.5 (3.1-9)	44	5.6 (4.5-8.1)	16	5.4 (4.5-6.5)	0.336
Urea, mmol/L	35	5.7 (3.7-9)	69	5 (1.9-9.9)	43	5.5 (1.8-8.9)	17	5.3 (3.1-9.3)	0.225
Full blood count									
Hb, g/dL	37	13.2 (10-16.5)	65	13.2 (10.4-15.4)	43	13.3 (10-3-16.7)	15	13.6 (10.1-15.3)	0.736
Platelets, $x10^9$/L	38	235.5 (101-456)	68	234 (109-405)	44	237 (109-461)	18	247 (116-395)	0.856
RBC, $x10^{12}$/L	38	4.4 (3.2-5.3)	67	4.3 (3-5.6)	43	4.5 (3-5.4)	18	4.5 (3.1-5.1)	0.201
Liver function tests									
ALT, µ/L	36	19 (8-73)	68	25.5 (11-91)	44	22 (10-62)	18	22 (13-55)	0.055
AP, µ/L	36	64 (32-109)	68	62 (29-770)	44	66.5 (29-123)	18	58 (47-139)	0.903
AST, µ/L	2	21.5 (13-30)	5	24 (20-45)	2	26.5 (18-35)	0	-	0.657
Bilirubin (total), µmol/L	36	11.5 (3-19)	69	12 (3-36)	44	11 (6-25)	18	10 (0-27)	0.332
GGT, µ/L	20	20.5 (1-155)	36	23.5 (10-250)	23	20 (9-70)	8	23.5 (8-440)	0.637
Protein levels									
Albumin, g/L	36	41 (23-49)	69	41 (26-48)	44	42 (35-48)	18	39.5 (32-46)	0.250
CRP, mg/L	31	2 (1-42)	63	2 (0-55)	42	1 (1-39)	18	1 (1-46)	0.801
Ferritin, ng/mL	2	64 (52-76)	5	99 (30-161)	1	38.5 (38.5-38.5)	0	-	0.465
Hormone levels									
TSH, µmol/L	34	1.5 (0.1-6.2)	64	1.4 (0.03-6.7)	40	1.4 (0.2-6.4)	17	1.8 (0.9-6.6)	0.434
Free T_4, pmol/L	34	14.3 (11.4-21.5)	64	14.4 (11.1-26.2)	39	14.5 (11.7-24.6)	16	12.8 (9.2-19)	0.051
Vitamin levels									
Vitamin A, µmol/L	16	1.8 (1.2-2.9)	37	2 (1.1-3.5)	22	1.8 (0.7-3.2)	7	1.7 (0.7-2.3)	0.256
Vitamin B_{12}, pg/mL	30	244 (133-998)	58	274.5 (119-934)	40	246.5 (114-701)	16	246 (101-522)	0.811

	n=	Definite SIBO n= 38	n=	Possible SIBO n= 70	n=	No SIBO n= 45	n=	Excluded n= 18	p-value
Vitamin D, nmol/L	20	45.5 (20-116)	39	45 (20-152)	25	53 (20-108)	7	63 (19-255)	0.860
Vitamin E, µmol/L	16	32.4 (22.2-43.9)	38	29.1 (16.9-62)	21	32.5 (19.4-45.2)	7	32.8 (20-39.3)	0.617
Red cell folate, ng/mL	33	443 (253-1,289)	56	512.5 (200-1,250)	38	472.5 (2131,236)	17	490 (192-1,185)	0.639
Serum folate, ng/mL	32	7.2 (2.5-725)	53	6.7 (1.9-596)	39	7.3 (2-25)	15	10.6 (1.6-25)	0.570
Trace elements									
Iron, µmol/L	34	12.5 (4-32)	62	14 (4-50)	40	14.5 (5-27)	17	11 (3-27)	0.523
Selenium, µmol/L	18	0.9 (0.7-1.4)	32	1.1 (0.2-1.6)	21	1.2 (0.5-1.7)	7	1.2 (0.7-3.5)	0.066
Zinc, µmol/L	16	13.8 (10.7-16.6)	29	12.8 (10.3-29.3)	19	12.4 (10.7-19.4)	5	13.7 (10-14.7)	0.964
Miscellaneous									
ESR, mm/hr	30	17 (4-57) [a]	40	15 (1-56)	29	10 (1-93) [a b]	13	30 (1-54) [b]	0.032 *
TIBC, µmol/L	34	53.5 (31-80) [c d]	61	58 (40-81) [e]	39	60 (40-81) [c]	17	61 (47-86) [d e]	0.012 *

Abbreviations: ALT, alanine transaminase; AP, alkaline phosphatase; AST, aspartate transaminase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GGT, gamma-glutamyl transpeptidase; Hb, haemoglobin; RBC, red blood cells; T_4, thyroxine; TIBC, total iron-binding capacity, TSH, thyroid-stimulating hormone.
Notes: Data expressed as median (range).
Kruskal-Wallis tests were performed to assess differences between the categories. Significant variables were adjusted for multiple comparisons. Post-hoc tests revealed that differences for ESR were between (1) Definite- and No SIBO categories (unadjusted: p= 0.022; adjusted: p= 0.131) (marked a) and (2) No SIBO and Excluded categories (unadjusted: p= 0.009; adjusted: p= 0.052) (marked b). The differences for TIBC were between (1) Definite- and No SIBO categories (unadjusted: p= 0.026; adjusted p= 0.153) (marked c), (2) Definite SIBO and Excluded categories (unadjusted: p= 0.002; adjusted: p= 0.011) (marked d) and (3) Possible SIBO and Excluded categories (unadjusted: p= 0.027; adjusted: p= 0.160) (marked e).

5.5.9 Binary Logistic Regression Analysis

Binary logistic regression was used to compare the 83 patients categorised as having Definite-SIBO (n= 38) and No SIBO (n= 45) to predict which variables were predictive of SIBO (Table 5-16). In the univariate setting, one variable was a significant predictor of SIBO at the p< 0.05 significance level: selenium (p= 0.045), where a one-unit (µmol/L) increase in the trace element decreased the risk of SIBO by 0.06 times. Also, previous treatment with radiotherapy/brachytherapy showed marginal significance (p= 0.05). For this variable, those who underwent previous radiotherapy and/or brachytherapy were three times less likely to develop SIBO than those individuals not receiving this treatment. The next strongest predictive variables (in order of strength) were total-iron binding capacity, tumour stage, prostate cancer and age, where the p-values were 0.056, 0.071, 0.090 and 0.131 respectively.

Table 5-16 Univariate odds ratio table for the prediction of small intestinal bacterial overgrowth in the 83 patients with Definite SIBO (n= 38) or No SIBO (n= 45)

Variables	n=	Categories	OR (95% CI)	p-value
Age (years)	83	Continuous	1.03 (0.99-1.07)	0.131
Gender	83	Female Male	1 1.39 (0.58-3.30)	0.458
Ethnicity	83	Other White	1 1.72 (0.15-19.75)	0.663
Primary tumour site	83	Gastrointestinal Urology Gynaecological Haematological Other	1 0.32 (0.10-0.98) 0.95 (0.27-3.31) 0.35 (0.03-4.42) 0.09 (0.09-2.90)	0.242
Prostate cancer	83	No Yes	2.24 (0.88-5.68) 1	0.090
Histopathological tumour (T) stage	64	0-2 3-4	1 0.37 (0.12-1.09)	0.071
Surgery	83	No Yes	1 1.96 (0.81-4.73)	0.135
Hormone therapy	79	No Yes	1.92 (0.76-4.89) 1	0.171
Chemotherapy	83	No Yes	1 1.37 (0.57-3.26)	0.479
Radiotherapy/ Brachytherapy	83	No Yes	3.00 (1.00- 8.99) 1	0.050
Creatinine	82	Continuous	1.00 (0.98 -1.02)	0.881
Glucose	79	Continuous	1.15 (0.72-1.84)	0.554
Urea	78	Continuous	1.11 (0.82-1.52)	0.503
Haemoglobin	80	Continuous	0.93 (0.68-1.26)	0.623
Red blood cells	81	Continuous	0.77 (0.31-1.87)	0.559
Albumin	80	Continuous	0.92 (0.83-1.03)	0.153

Variables	n=	Categories	OR (95% CI)	p-value
Vitamin B_{12}	70	Continuous	1.00 (0.99-1.00)	0.859
Selenium	39	Continuous	0.06 (0.00-0.93)	0.045 *
ESR	59	Continuous	1.03 (0.99-1.07)	0.169
TIBC	73	Continuous	0.95 (0.90-1.00)	0.056
Abbreviations: ESR, erythrocyte sedimentation rate; TIBC, total iron-binding capacity Notes: * p< 0.05 The Box-Tidwell test was conducted to test the assumption that the relationship between the continuous predictors and the logit (log odds) was linear: tests revealed that the assumption was not violated for any of the continuous predictors.				

5.5.10 New Gastrointestinal Diagnosis

As each recruited patient was managed using a peer-reviewed investigation and treatment algorithm, they generally underwent multiple investigations, so as to determine the cause for their GI symptoms. As such, apart from SIBO, patients may have received other GI diagnoses between the baseline and follow-up visits, as described in Table 5-17. There were more new diagnoses in those with Possible SIBO (60%) and No SIBO (58%) as compared with Definite SIBO (50%). In those with Definite SIBO, 26.3% were also diagnosed with bile acid malabsorption.

Table 5-17 New gastrointestinal diagnoses other than small intestinal bacterial overgrowth for the 171 patients with a diagnostic category

n (%)	Definite SIBO n= 38	Possible SIBO n= 70	No SIBO n= 45	Excluded n= 18
No	19 (50)	28 (40)	19 (42.2)	12 (66.7)
Yes *	19 (50)	42 (60)	26 (57.8)	6 (33.3)
Bile acid malabsorption	10 (26.3)	26 (37.1)	16 (35.6)	3 (16.7)
Gastritis	3 (7.9)	3 (4.3)	2 (4.4)	2 (11.1)
Pancreatic insufficiency	3 (7.9)	4 (5.7)	0 (0)	0 (0)
Inflammatory changes	0 (0)	2 (2.9)	3 (6.7)	0 (0)
Coeliac disease	0 (0)	1 (1.4)	1 (2.2)	0 (0)
Motility disorder	1 (2.6)	0 (0)	0 (0)	1 (5.6)
Drug-related issues	0 (0)	1 (1.4)	2 (4.4)	0 (0)
Bile acid reflux	0 (0)	2 (2.9)	0 (0)	0 (0)
Infection	1 (2.6)	2 (2.9)	0 (0)	0 (0)
Hiatus hernia	0 (0)	2 (2.9)	0 (0)	0 (0)
Diverticular disease	2 (5.2)	0 (0)	0 (0)	0 (0)
Oesophageal lesion	0 (0)	0 (0)	2 (4.4)	0 (0)
* One patient in each of the Definite SIBO and Possible SIBO categories had two diagnoses				

5.5.11 Primary Outcome: Metabolite Levels in Baseline Urine Samples Using ^{1}H NMR

Hydrogen Nuclear Magnetic Resonance was performed on 196 baseline urine samples: samples were available for all patients in the Definite SIBO (n= 38) and No SIBO (n= 45) categories. Principal component analysis and PLSDA were performed on all of the spectral variables, with no separation of the categories noted. Single variable analysis was performed using independent t-testing. Following this, using only the variables that had a p-value of < 0.05 (after correcting for multiple testing), PCA was performed again. There was some, albeit not distinct separation of the categories noted, as shown in Figure 5-8. A support vector machine classifier was used to predict which points on this PCA plot belonged to the Definite SIBO category and which belonged to the No SIBO category, with good discriminatory ability noted.

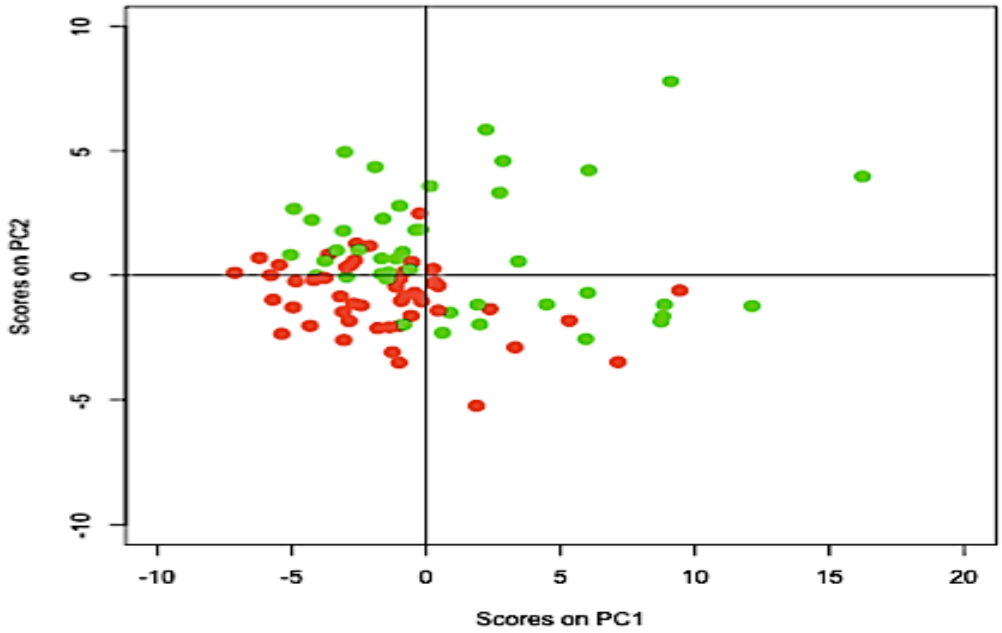


Figure 5-8 Principal component (PC) analysis plot derived from the 700 MHz ^{1}H NMR spectra of baseline urine samples. Comparison of the mean metabolic PC1/PC2 trajectories: Definite SIBO, green; No SIBO, red

Following Adaptive Intelligent Binning, there were 68 bins deemed possible candidates for being significant variables (p-values were < 0.05) in the separation of the two diagnostic categories. Each bin was ranked according to statistical significance with the bin ranked number 1 having the lowest (most significant) p-value (Table 5-18). Individual spectra were visualised to determine the presence of *'real'* peaks in the region of these 68 bins, rather than *'noise'*

variables (which are insignificant). After the elimination of *'noise'* variables and considering the proximity of bins to one another on a spectrum, the following five bins were considered worthy of further investigation: Bins 264, 265, 266, 267 and 268, as highlighted in Table 5-18.

Table 5-18 Results of Adaptive Intelligent Binning performed on the spectra of urine samples from Definite SIBO and No SIBO patients

Bin number	p-value	Rank	Lower ppm	Upper ppm
21	0.050	68	8.4496	8.4302
23	0.042	54	8.3842	8.366
44	0.033	38	8.0834	8.0751
53	0.010	11	7.9865	7.9756
63	0.048	67	7.8882	7.876
71	0.044	59	7.7843	7.7706
72	0.038	43	7.7706	7.749
73	0.021	26	7.749	7.7329
94	0.015	15	7.4267	7.4162
95	0.011	12	7.4162	7.4056
96	0.016	16	7.4056	7.3945
103	0.036	41	7.3279	7.3167
108	0.021	25	7.2783	7.2672
117	0.040	44	7.1895	7.1815
118	0.030	34	7.1815	7.1772
138	0.040	47	7.0035	6.9959
140	0.040	46	6.9913	6.9837
142	0.041	49	6.9752	6.9677
154	0.018	22	6.8713	6.8583
155	0.043	57	6.8583	6.8531
165	0.030	36	6.765	6.7114
179	0.036	40	6.5555	6.5287
264	0.016	17	4.1954	4.1845
265	0.005	4	4.1845	4.1789
266	0.007	5	4.1789	4.1733
267	0.018	24	4.1733	4.1664
268	0.004	3	4.1664	4.1615
269	0.007	6	4.1615	4.1525
355	0.017	19	3.6662	3.6563
358	0.042	51	3.6454	3.6398
359	0.047	64	3.6398	3.6366
399	0.043	55	3.4057	3.4013
431	0.025	31	3.2016	3.1962

Bin number	p-value	Rank	Lower ppm	Upper ppm
437	0.031	37	3.1601	3.1523
454	0.034	39	3.0503	3.0428
459	0.008	7	3.0112	3.0053
460	0.003	2	3.0053	3.0002
467	0.010	10	2.9465	2.9353
482	0.042	52	2.8315	2.8251
502	0.026	32	2.6367	2.6267
504	0.040	45	2.6151	2.61
507	0.017	21	2.5995	2.5962
509	0.041	50	2.5921	2.586
522	0.041	48	2.4896	2.4858
544	< 0.001	1	2.3236	2.3197
546	0.042	53	2.3136	2.3039
552	0.013	14	2.2745	2.264
554	0.010	9	2.261	2.2494
555	0.008	8	2.2494	2.2364
557	0.048	66	2.2291	2.2241
574	0.047	63	2.1087	2.0998
575	0.022	28	2.0998	2.096
576	0.016	18	2.096	2.0896
577	0.025	30	2.0896	2.0835
578	0.047	65	2.0835	2.0775
596	0.017	20	1.9468	1.9377
597	0.046	62	1.9377	1.9305
598	0.043	58	1.9305	1.9251
602	0.030	35	1.9035	1.8906
611	0.038	42	1.8181	1.8095
614	0.045	61	1.7926	1.7842
626	0.022	27	1.7085	1.6967
627	0.013	13	1.6967	1.689
650	0.044	60	1.4568	1.4504
674	0.043	56	1.2946	1.2848
686	0.023	29	1.1893	1.1844
698	0.018	23	1.1027	1.0917
699	0.029	33	1.0917	1.0802

Abbreviation: Ppm, parts per million
Note: Bins 264, 265, 266, 267 and 268 (shaded green) underwent further analysis

Focusing on these five bins, the ^{1}H NMR spectra for all Definite SIBO and No SIBO samples were stacked one on top of another to display the spectral area that encompassed them, as shown in Figure 5-9. Within this area, there was a multiplet (an NMR signal that is split) at 4.20-4.15 ppm, containing six peaks (sextet), which appeared to be causing separation of the two categories. An example spectrum (from a Definite SIBO patient) containing the highest level of this multiplet is shown in Figure 5-10.

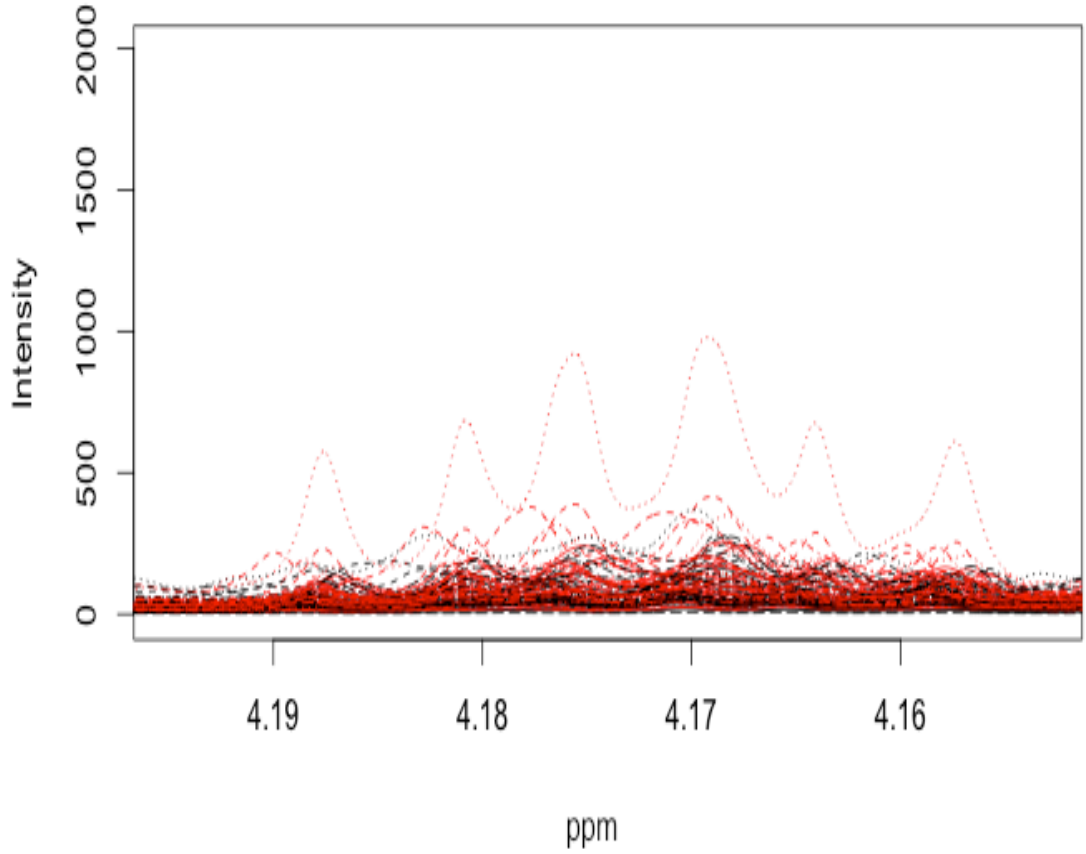


Figure 5-9 700 MHz ^{1}H NMR spectra of baseline urine samples of Definite SIBO (red spectra) and No SIBO (black spectra) patients zoomed into the spectral area of interest containing the multiplet: the highest intensity of the multiplet can be identified in Definite SIBO samples

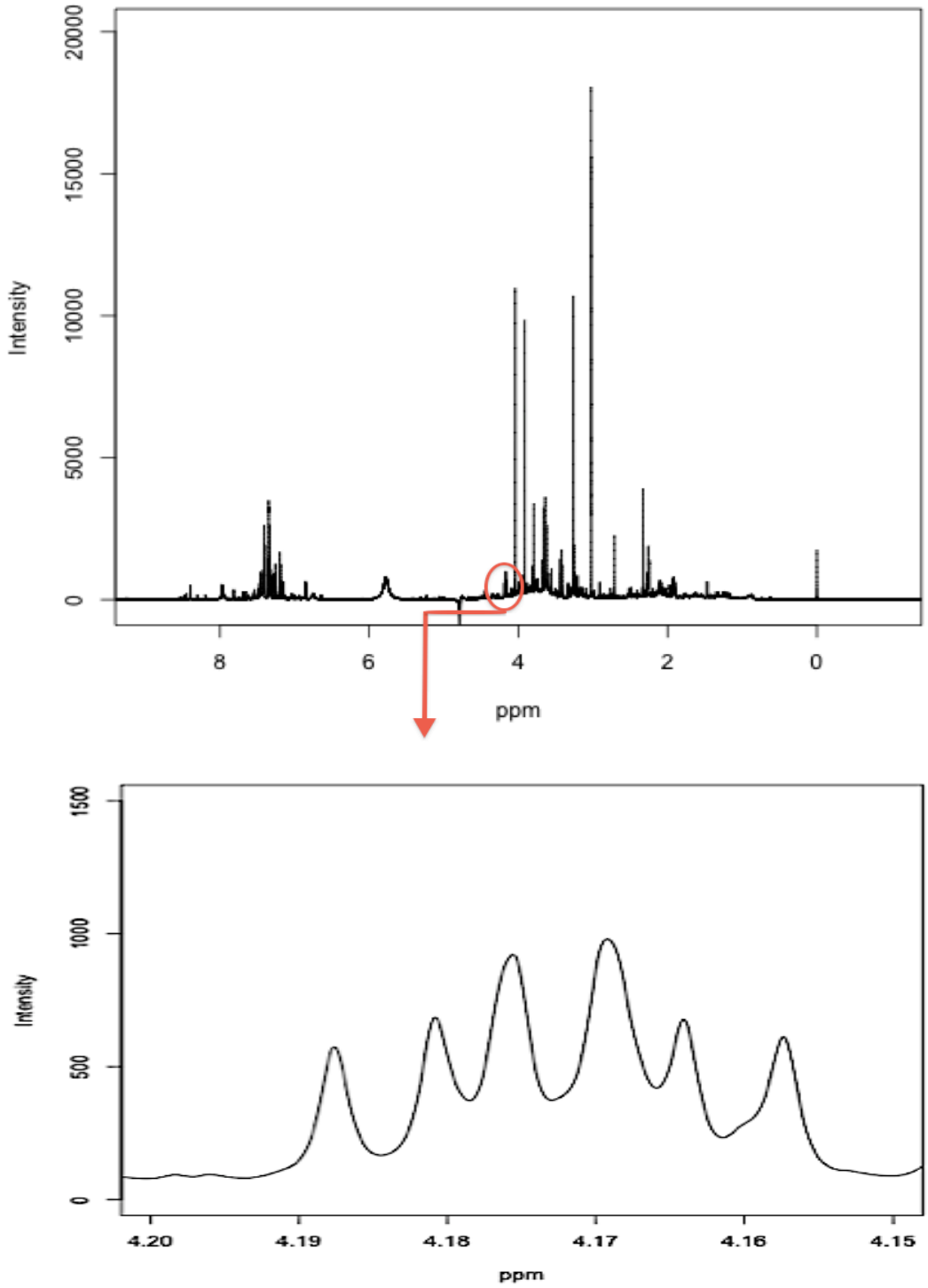


Figure 5-10 700 MHz ^{1}H NMR spectrum of the baseline urine sample from a Definite SIBO patient with the highest intensity of the multiplet at 4.20-4.15 parts per million: top figure shows the full spectrum; bottom figure shows the area of interest zoomed into

The *k*-Nearest Neighbours algorithm was used for classification of the two categories using Bin 268 from this multiplet, as it had the smallest p-value of the five bins. As shown in Figure 5-11, when all of the samples in Definite SIBO and No SIBO were combined, 11 of the top 12 highest spectral intensities were from the Definite SIBO category. To test Bin 268 further, a small amount of noise (jitter) was added to the numeric vector, which prevents observations with the same spectral intensity from covering each other up. The resulting 1D beeswarm jitter plot also shows relatively good separation of the patient categories (Figure 5-12).

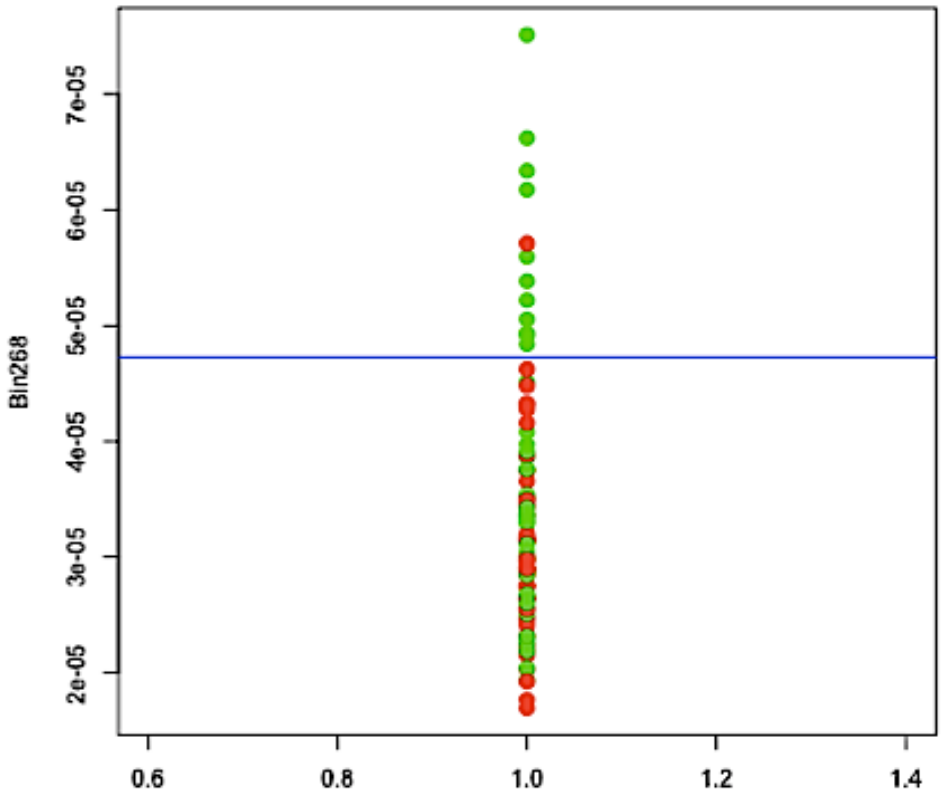


Figure 5-11 Classification of the Definite SIBO patients (green dots) and No SIBO patients (red dots) with respect to intensity of the signals within Bin 268 using a *k*-Nearest Neighbours plot

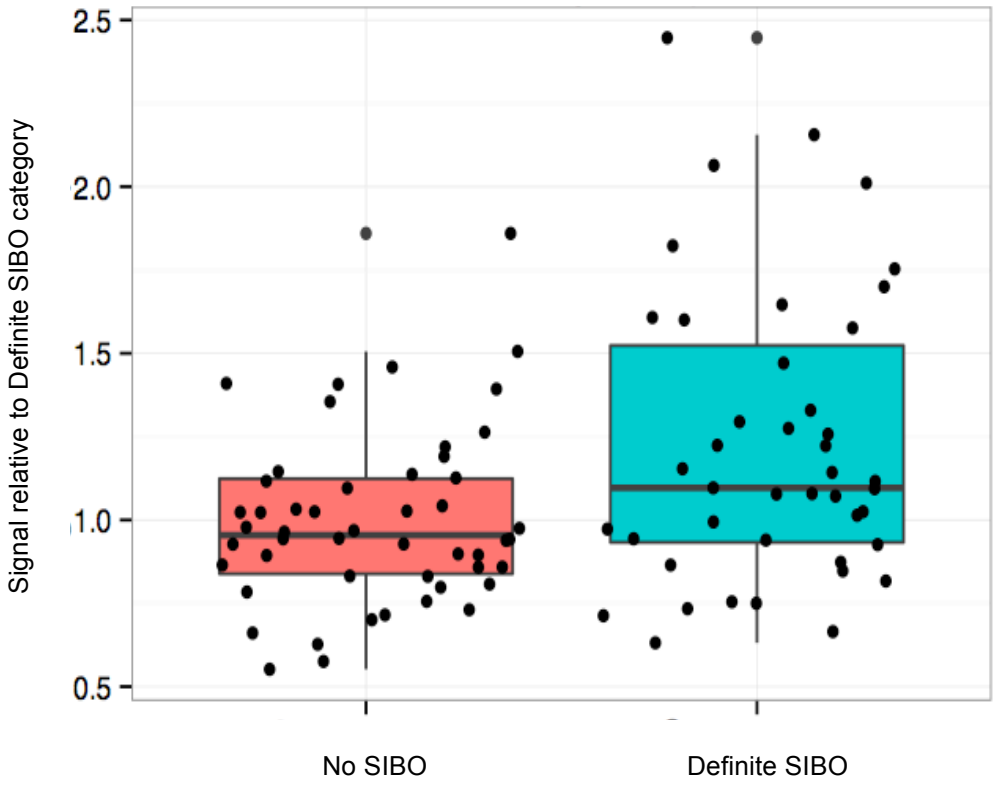


Figure 5-12 Beeswarm jitter plot derived from the classification of Definite SIBO and No SIBO by the intensity of the signals within Bin 268

The 2D spectrum of one sample (i.e. the one with highest intensity of signals within the five bins) was analysed so as to clarify the structure of the metabolite(s) found within that region of the spectrum. The peaks in the spectrum correspond to a compound called N-acethylglutamine (Figure 5-13). The chemical structure of N-acethylglutamine is displayed in Figure 5-14.

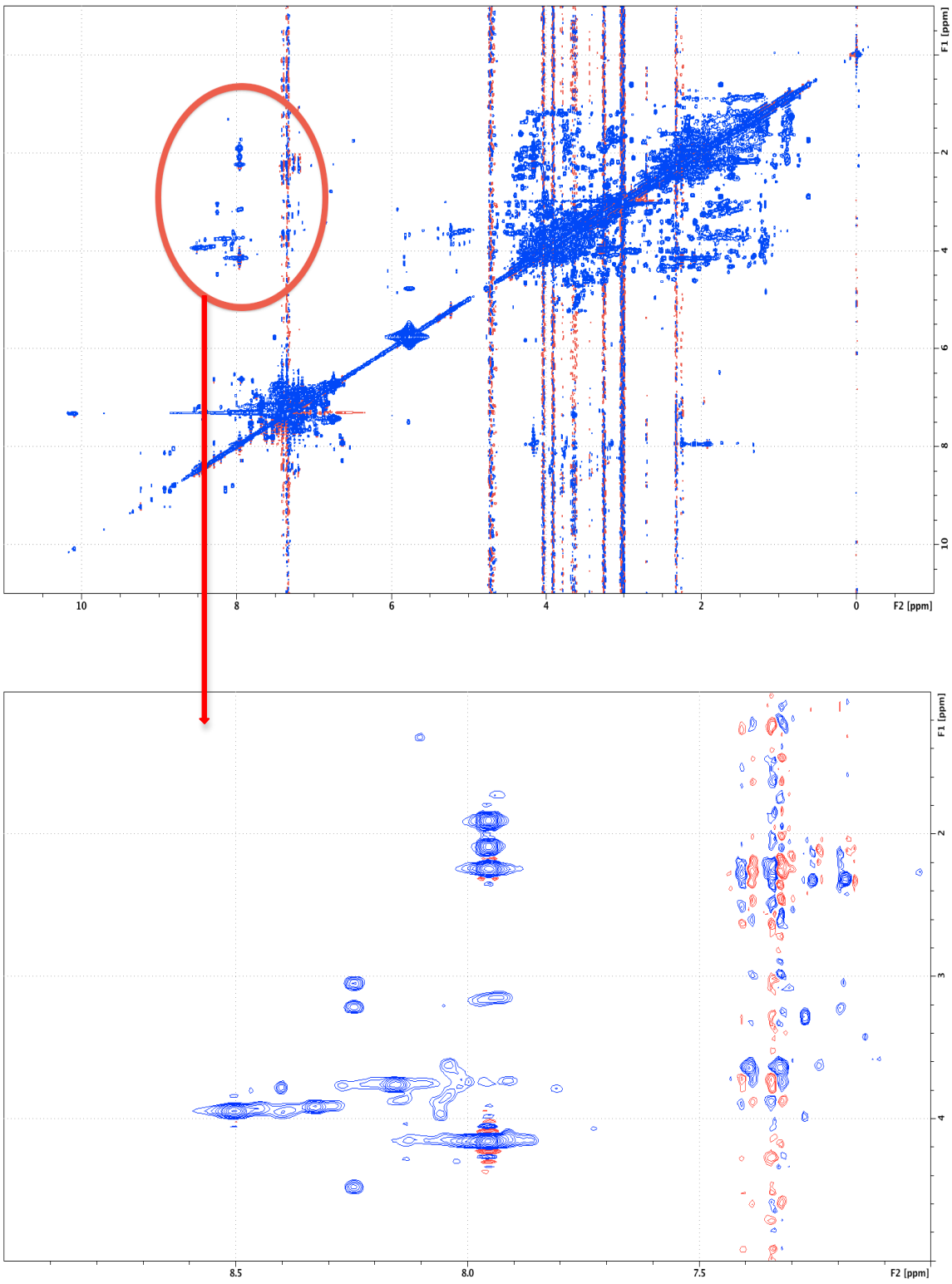


Figure 5-13 ^{1}H NMR 2D spectrum: the structural pattern of N-acethylglutamine is zoomed into in the bottom figure

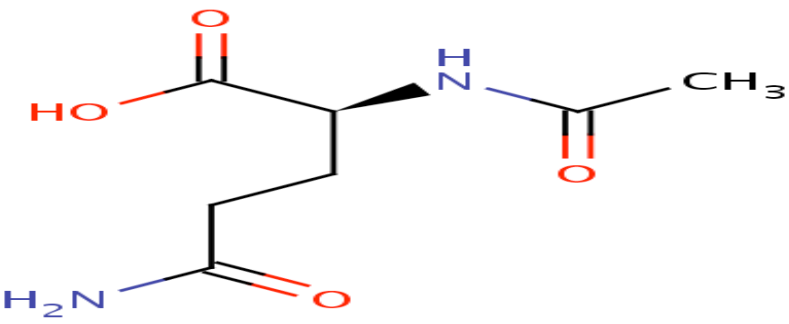


Figure 5-14 Chemical structure of N-acethylglutamine: $C_7H_{12}N_2O_4$

5.5.11.1 Hypothesis 1: Metabolomics Will Indicate the Presence or Absence of Small Intestinal bacterial Overgrowth

This study's hypothesis was that qualitative and quantitative analyses of metabolites in urine would indicate the presence or absence of SIBO in patients previously or currently being treated for cancer. Using selected data modeling techniques, the results available thus far suggest that metabolomics may have some ability to separate patients with SIBO from those without it based on the metabolite N-acethylglutamine. However, the concentration of N-acethylglutamine was not consistently higher in those with SIBO compared with those without SIBO. Therefore, the compound does not yet appear sensitive or specific enough to indicate the presence or absence of SIBO. Thus, this research hypothesis cannot be accepted at this stage. Further research is required in order to investigate whether this compound, or others, would be suitable candidate metabolites to discriminate between individuals with and without SIBO.

5.6 Discussion

5.6.1 Small Intestinal Bacterial Overgrowth Characteristics

This is the first prospective study to measure the prevalence of SIBO in a cohort of patients previously diagnosed with cancer and reporting the new onset of GI symptoms. In this study, the prevalence of Definite SIBO was 22.2%, with a further 26.5% also potentially having the condition (i.e. Possible SIBO). Previous retrospective data from RM (n= 435) suggested that the prevalence of SIBO in cancer was somewhat lower; 11% were found to have Definite SIBO and

17% had Possible SIBO (unpublished data). The baseline characteristics of that cohort were very comparable to the current study's cohort: 50% were male; the median age of the males and females was 69 (22-89) and 59 (28-90) years respectively; 31% had GI cancer, 32% urological, 27% gynaecological, 4% haematological and 6% had another cancer type. Considering the categorisation of patients was done in a comparable way for both studies, one can surmise that the true prevalence of SIBO may have been higher in the retrospective study had there been less unavailable data (46% could not be assessed for SIBO status due to missing data).

While there are no other studies, retrospective or otherwise, of SIBO in such a diverse cohort of cancer patients, one prospective observational study investigated SIBO in a mixed cohort of GI, gynaecological and urological cancer patients undergoing pelvic radiotherapy (Wedlake et al. 2008). In this study, 38 patients underwent a glucose-H_2 breath test before commencing radiotherapy and again after four-five weeks of treatment. Of these patients, 10 (26%) had a positive breath test at the follow-up time point, after having had a negative baseline test. The design of this pilot study was not akin to the current study: assessment of SIBO was done in the acute phase of cancer therapy; direct aspiration and quantification of jejunal fluid was not undertaken; and patients' GI symptoms were not assessed following antibiotic treatment. Nonetheless, the prevalence of SIBO appears to correspond to that reported in the current study (22.2%).

One of the objectives of this investigative study was to identify the variables that are characteristic of SIBO. It is the first study to measure an extensive number of both upper- and lower-GI symptoms systematically in patients undergoing testing for SIBO. At baseline, in those with Definite SIBO, the median (range) number of symptoms reported was 13.5 (5-26), which highlights the burden of the condition. In this regard, the most commonly reported symptoms were flatulence, faecal urgency, loose stools, abdominal grumbling and incomplete evacuation, ranging from 84-97%. Other notable symptoms included belching, nausea, early satiety, bloating, abdominal pain, diarrhoea, constipation, and faecal incontinence found to occur in 47-

79% of those with Definite SIBO. These results show some parallels with the literature. Diarrhoea, abdominal pain and constipation in this study were at the upper-end of the prevalence figures reported in previous prospective and retrospective studies: 79% (literature: 13-89%), 74% (literature: 13-86%) and 53% (literature: 10-77%) respectively. The prevalence of bloating in the current study (68%) was higher than previously reported (10-50%) (Lombardo et al. 2010; Tursi et al. 2003; Marie et al. 2009; Compare et al. 2010; Pimentel et al. 2000; George et al. 2012; Davidson et al. 1984; Roberts et al. 1977). However, analysis was not undertaken to determine if individual symptoms were significantly higher in those with Definite SIBO compared with No SIBO, so these data should be interpreted with caution.

Remarkably, the current study appears to be the first to recognise the following symptoms associated with SIBO: faecal urgency (reported in 92%), abdominal grumbling (89%), incomplete evacuation (84%), belching (79%), faecal incontinence (68%) and early satiety (50%). This indicates that the GI characteristics of SIBO have not been fully understood due to consistently poor GI symptom assessment to date. However, on a positive note, this study demonstrates an improvement in GI function following the treatment of antibiotics in those with Definite SIBO: median (range) number of symptoms was 13.5 (5-26) at baseline and 3 (0-10) at follow-up; median (range) GSRS total score was 23.5 (7-52) at baseline and 19 (4-53) at follow-up. In addition, patients reported less loose stools following treatment.

In addition to measuring the symptoms associated with SIBO, this study also assessed biochemistry and haematological variables in the cohort. Early animal studies suggested that SIBO may be associated with reduced haemoglobin levels as a result of GI bleeding within a blind loop (Giannella & Toskes 1976). More recently, in a study of 51 patients with systemic sclerosis, those with SIBO had significantly lower median levels of haemoglobin than those without it (12.25 vs. 13.9 g/dL, p= 0.002) (Marie et al. 2009). Similarly, in a study of 43 gastrectomised patients, haemoglobin concentration tended to be lower in the patients with a maximum H_2 concentration in the glucose-H_2 breath test (p= 0.056) (Iivonen et al. 1998). However, the current study does not support these previous data: the median (range)

haemoglobin level of those with SIBO (13.2 (10-16.5) g/dL) was neither significantly different to those without SIBO nor low according to standard reference ranges (Thomas & Bishop 2007). Theoretically, SIBO may be associated with raised total iron-binding capacity (marker of inflammation), but in the current study, Definite SIBO was not associated with significantly higher levels of it as compared with No SIBO (after adjusting for multiple comparisons).

Vitamin B_{12} has also received some attention with regard to SIBO, as facultative Gram-negative aerobes and anaerobes have been shown to be capable of competitively utilising vitamin B_{12} (Welkos et al. 1981). However, data with regard to deficiency of the vitamin have proven inconsistent to date. For example, a study of 16 elderly patients with confirmed SIBO (as per lactulose-H_2 breath test) reported that 10 (62.5%) had subnormal serum vitamin B_{12} levels (Haboubi & Montgomery 1992). Conflictingly, in the aforementioned systemic sclerosis cohort, no significant difference in vitamin B_{12} levels was noted between patients with and without SIBO ($p= 0.133$) (Marie et al. 2009). Also, in a 35-patient study (n= 22 with GI disease, n= 13 controls), there was no significance difference in vitamin B_{12} levels between those with and without SIBO (SIBO was confirmed using the direct aspiration method) (Hamilton et al. 1970). The current study supports the latter two, with no significant difference noted between those with Definite- or No SIBO with regard to vitamin B_{12} and a median (range) value of 244 (133-998) pg/mL noted for those with Definite SIBO.

Fat-soluble vitamin status has been explored in SIBO as intraluminal bacteria have been shown to deconjugate bile acids possibly causing malabsorption of fat and fat-soluble vitamins (Shindo et al. 1998). However, to date, there have only been case-reports of vitamin E deficiency syndromes (neuropathy, T-cell abnormalities) and night blindness caused by vitamin A deficiency in patients with SIBO (Brin et al. 1985; Kowdley et al. 1992; Hasan & Finucane 1993). Similarly, although vitamin D deficiency is believed to be a complication of SIBO, there have been few data strongly demonstrating this to be the case (Stotzer et al. 2003). With regard to these fat-soluble vitamins, the current study does not indicate that their levels are lower in those with SIBO, as compared with the other patient categories, nor below normal reference

ranges (Thomas & Bishop 2007). In fact, there were no biochemistry or haematological variables that appeared to be abnormal in patients with Definite SIBO. This suggests that, contrary to common belief, there may be no suitable non-invasive surrogate markers of SIBO.

As discussed, there is no consensus on the optimal test for use in detecting SIBO. In the current study, two commonly used approaches were utilised- the GHMBT and jejunal aspiration with microbiological quantification. There were 153 patients for whom results were available for both tests. Interestingly, concurrent results for the two tests occurred in just 58.2% of these patients. If the direct aspiration method was taken to be the gold-standard, as was traditionally the case (although not justifiably), 47 (30.7%) of the GHMBTs would be considered false positives and 17 (11.1%) would be considered false negatives.

These results can be compared with the findings of a review of different cross-validation studies between the glucose-H_2 breath test and jejunal aspirate culture. The reviewers aimed to determine the diagnostic accuracy of the breath test for SIBO by taking the jejunal aspirate to be the gold-standard (Gasbarrini et al. 2009). Although, the eight studies were very heterogeneous, the authors determined that the glucose-H_2 breath test had a sensitivity of 62.5%, a specificity of 81.7%, a positive predictive value of 80% and a negative predictive value of 65.5% compared with the gold-standard. Therefore, because the sensitivity is not high, it is unlikely that the breath test overestimated SIBO (i.e. produced false positives) in the current study, as appears to be the case. Rather, one can assume that the lack of appropriate anaerobic microbiological techniques at RM may have caused the overgrowth of anaerobic organisms to be missed, thus making it seem that the GHMBT produced high numbers of false positive results. Though, of course, one cannot be certain on this, as the review was specific to the glucose-H_2 breath test, not the GHMBT and the sensitivity and specificity of the latter has not yet been estimated. However, the addition of CH_4 should, in theory, make the test more sensitive, as it can detect CH_4 in the subgroup of H_2 non-producers (2-43%) (Cloarec et al. 1990; Saltzberg et al. 1988; Read et al. 1985; Joseph & Rosenberg 1988; Gilat et al. 1978; Bjorneklett & Jenssen 1982; Flatz et al. 1985; Corazza et al. 1994). Therefore, some of the perceived false positives in the

current study may be actual false positives, and some may have been a spurious finding caused by poor anaerobic laboratory methods. This emphasises the inconsistency between the testing techniques and the need for a true gold-standard test for the diagnosis of SIBO.

One of the objectives of this study was to identify the variables that are predictive of SIBO. Following univariate analysis, selenium showed significance (0.045), OR (CI): 0.06 (0.00-0.93). Selenium is an essential trace element, primarily absorbed in the duodenum (Thomas & Bishop 2007). It has a key role in antioxidant defence, thyroid hormone metabolism and immune function, with growing evidence to suggest that selenium deficiency affects both cell-mediated and humoral components of the immune response (Fairweather-Tait et al. 2011; Speckmann & Steinbrenner 2014). While there have been no previous data suggesting that higher plasma selenium levels decreased the risk of SIBO, one can speculate why this may be the case. Patients with SIBO are known to have abnormalities in intestinal mucosal immunity, which could potentially be associated with selenium insufficiency, although this has not yet been investigated (Riordan et al. 1999; Riordan, McIver, Wakefield, et al. 1997b). Also, there are animal data indicating that dietary selenium intake affects the gut microbiota, and *vice versa*, but this has not yet been studied in the context of SIBO (Hrdina et al. 2009; Kasaikina et al. 2011). Because of its antioxidative effects, selenium has received considerable attention with regard to cancer prevention and treatment, particularly for prostate cancer (Chen et al. 2013; Mandair et al. 2014). Given that 26% of those with Definite SIBO had a prostate cancer diagnosis, selenium's significance in the univariate model may have been an artifact of selenium supplementation by these men. Also, individuals with diarrhoea are likely to have lower serum selenium due to losses of the trace element in diarrhoea and this may offer an alternative explanation for selenium being a significant variable. Nonetheless, one should be cautious when interpreting the OR for selenium as data was missing in 53% of cases.

Radiotherapy/brachytherapy showed marginal significance in the univariate model, p= 0.05, OR (CI): 3.00 (1.00-8.99). In effect, those patients that did not undergo radiotherapy and/or brachytherapy were three times more likely to develop SIBO than those individuals receiving

this treatment. This is an unexpected finding, considering the aforementioned 38-patient study, which reported a SIBO incidence of 26% in pelvic radiotherapy patients (Wedlake et al. 2008). In addition, Husebye et al. recruited 41 consecutive female patients with symptoms of late radiation enteropathy following gynaecological cancer (1995). Following duodenal aspiration and quantification, microorganisms were detected in samples from 71% of patients and Gram-negative bacilli were detected in 29%. Intestinal motor patterns were also investigated in this cohort, and the results suggested that the chronic motility changes caused by radiotherapy were the main cause for SIBO. The data suggested a high prevalence of SIBO following pelvic radiotherapy, and a case-report also suggested that radiotherapy was the cause for SIBO in a gynaecological patient (Swan 1974).

The disagreement between the current study and the Wedlake and Husebye studies may be caused by the heterogeneity of the cohorts and the time of testing for SIBO. Husebye et al. included women with long-term GI complaints following treatment for gynaecological cancer, where the median (range) time since end-treatment was 10 (1-43) years. Wedlake et al. included patients undergoing pelvic radiotherapy for GI, gynaecological or urological cancer in the acute setting. The current study recruited patients with GI, gynaecological, urological, haematological cancers, lymphomas and other cancer types, where the median (range) time lapsed since treatment completion was 35.5 (0-512) months. Effectively, the current study offers neither an acute nor a chronic picture of SIBO in cancer, rather somewhere in between the two. In addition, it must be borne in mind that injury to the GI tract depends on the type of radiotherapy given, the dose delivered to tissues, the way it is delivered, and how radiation energy scatters through tissues, variables that were not measured in any of the three studies discussed.

5.6.1.1 Strengths and Limitations of SIBO Methods and Results

This is the first large prospective study to investigate the characteristics of SIBO in patients with cancer. However, there are some inherent limitations associated with the study design. Generally with a case-control study, one identifies subjects by outcome status at the outset of

the investigation. Once outcome status is identified and subjects are categorised as cases, controls (subjects without the outcome but from the same source population) are selected. In the current study, the approach was different in that it was unknown which patients would become cases and which would become controls at the study outset. This is because patients were selected from the same gastroenterology clinics, where they presented with similar GI symptoms. This sampling method means that the control group is not ideal for this case-control study, although they do represent a clinically relevant group, as the differences detected in baseline characteristics and laboratory variables detected here are important. However, had an appropriate age- and sex-matched healthy control group been used, there would almost certainly have been greater differences in baseline characteristics and GI symptoms between the categories.

The control of extraneous variables, in particular with regard to interventions for other GI conditions, was considered when categorising patients, leading to the exclusion of 18 patients and the classification of others as having Possible SIBO. However, one cannot be certain that confounding was not involved in more of the Definite SIBO cases, particularly as half of this category was also concurrently diagnosed with another GI condition. As such, it is not possible to be confident that the GI symptoms reported at baseline by those with Definite SIBO are solely the consequence of overgrowth. Also, if the improvement in GI symptoms was related to the treatment of another GI condition (not SIBO), this may have led to the misclassification of patients in the Definite SIBO category. To correct for this, one could have ensured that patients were not treated for any other condition (apart from SIBO) during the study. This would have created a cleaner cohort but because of the high symptom burden experienced by these patients, such a decision would have been unethical. Alternatively, it would have been appropriate to record the proportion of patients who received interventions, other than antibiotics for SIBO, during the study.

Potential bias may have been caused by the large differences in the length of time between baseline and follow-up; some (4%) were followed-up < 4 weeks after baseline, while 61% were

followed-up > 12 weeks after baseline. Where there was a longer time period between the study visits, there may have been a (a) higher chance that the patient commenced treatment for another GI condition and (b) longer post-antibiotic treatment period before GI symptoms were re-assessed, in comparison to a shorter follow-up period. The variability in the time lapsed between study visits was partly due to the decision to only see patients for study purposes on the day of another routine appointment. However, this attempt to avoid unnecessary hospital visits may have introduced bias caused by the lack of a robust approach towards the timing of follow-ups.

Although the approach used in this study to categorisation patients (in terms of SIBO) was the consensus of a multi-professional discussion, one can question its robustness. Firstly, the diagnosis of SIBO by the gastroenterologist was based largely on the improvement in GI symptoms following antibiotic treatment, as measured by the GSRS tool. It has previously been noted that this symptom tool was a modified version of the original tool and was not validated for use in a cohort of cancer patients with suspected SIBO. Secondly, reporting bias needs to be considered as patients may have falsely reported an improvement in symptoms in the belief that this is what the researcher wished to hear. Thirdly, by virtue of the fact that these unwell patients were undergoing investigations and interventions for their GI problems, this may have improved emotional and psychological well-being. As a result, the perceived severity of symptoms may have been less than the actual reality at follow-up.

The introduction of an objective element to the follow-up visit would have helped to remove these biases. For example, if every patient deemed to have Definite SIBO underwent a repeat GHMBT, and the test was negative, Definite SIBO could then be confirmed. This approach may also have helped to reduce the size of the Possible SIBO category, as some of these patients were categorised as such because it was unsure if the positive clinical response exhibited was produced by factors other than antibiotics. However, in practice, an extra breath test would require considerably more resources (money and time) and the willingness of patients to

undergo a second test, with all that this entails. Also, repeating the test would not actually change the patient's management as they had already received antibiotic treatment.

Missing data represents a challenge to most research studies, including this one. In the current study, the inability to fit a multivariate model for SIBO prediction was a consequence of missing data, as well as small numbers within the Definite- and No SIBO categories. There were just 38 patients in the Definite SIBO category, which is 28 less than the number used to power the study. Although, every effort was made to ensure data completeness, this was not always achievable. For instance, the number and type of biochemistry and haematological tests requested for patients was not always consistent, as this depended upon the patient's GI symptoms and differential diagnoses (Andreyev et al. 2014). Also, the majority of the recruited cohort was referred from the primary or secondary care setting to RM and as such their full medical history was not always available. If the study dietitian had requested additional biochemistry and haematological tests when needed, and contacted the patient's previous caregiver for additional medical records, this would have reduced the amount of missing data. Alternatively, the recruitment of more patients would have ensured that the categories were bigger, which would have alleviated the problem of over-fitting (i.e. random error or noise instead of a true underlying relationship) in the multivariate setting.

5.6.2 Primary Outcome

The primary objective of this pilot study was to generate valid data on a wide range of metabolites present in the urine samples of patient who underwent testing for SIBO to determine if there was any metabolite(s) that could separate those with SIBO from those without it. The obtained data suggest that there is an up-regulation of N-acetylglutamine production in patients with SIBO. N-acethlyglutamine is a modified amino acid, which activates the carbamoyl phosphate synthase in the urea cycle. It is biosynthesised from glutamic acid and acetyl-CoA by the enzyme N-acetylglutamate synthase. It is an acetylated analogue and pre-curser of glutamine- a substrate which is the major fuel for enterocytes (Bergana et al. 2000). In fact, N-acetylglutamine has been considered as a source of glutamine in both enteral and parenteral

nutrition (Magnusson et al. 2013; Arnaud et al. 2004). It is a metabolite which is located on the surface of tubular cells and has been detected in the urine of healthy individuals (Sugahara et al. 1994; Sachse et al. 2012).

There are some conditions that have been associated with the up-regulation of N-acetylglutamine. For example, researchers investigating lung cancer metabolic signatures in urine (using ^{1}H NMR-based metabolomics) demonstrated metabolic differences between controls (n= 54) and cancer subjects (n= 71) (Carrola et al. 2011). It was found that N-acetylglutamine was consistently elevated in patients with cancer compared with controls. Similarly in a study of autosomal dominant polycystic kidney disease, significantly elevated levels of N-acetylglutamine were detected in the urine of cases (n= 54) as compared with controls (n= 46) (Gronwald et al. 2011). Raised levels have also been linked to low estimated glomerular filtration rates in non-proteinuric patients with type 2 diabetes (Ng et al. 2012). However, the cause for the elevation in N-acetylglutamine has not been elucidated in any of these three patient groups.

The Human Metabolome Database, which is the complete and comprehensive curated collection of human metabolite and human metabolism data in the world, does not indicate that increased concentrations of N-acetylglutamine are associated with any disorder of the GI tract (Wishart et al. 2013). As such, one can only speculate as to the reason for elevated levels of this metabolite in the urine of individuals believed to have SIBO. As mentioned, N-acetylglutamine is biosynthesised from glutamic acid, which is a component of the cell-wall complex of Gram-positive bacteria. The degradation of these bacteria would release glutamic acid, making it available for N-acetylglutamine production, leading to its appearance in urine. Alternatively, high urinary excretion of this compound could potentially be the result of bacterial synthesis of it, leading to enzyme system saturation, and thus, the removal of the excess in urine. However, to date, there have been no reports suggesting that N-acetylglutamine is a bacterial metabolite. Instead, it is possible that N-acetylglutamine is a marker of cancer, considering it was found to be consistently elevated in patients with lung cancer compared with

controls (Carrola et al. 2011). In support of this, a metabolomics study of colorectal cancer patients found that those with cancer had higher serum levels ($p < 0.05$) of N-acetyl signals from glycoproteins compared with controls (Bertini et al. 2012).

The current study was performed with no *a priori* information regarding the composition of the urine samples in patients with suspected SIBO (i.e. it was untargeted). Thus, it can be considered a hypothesis-generating study, where a metabolite of interest was discovered. The discovery of N-acetylglutamine represents just the first of many steps needed before a compound could be considered a biomarker of SIBO. In the current study, a second urine sample was collected from patients after the completion of antibiotic treatment. Therefore, the most appropriate next step would be to establish whether antibiotic treatment resulted in a lower spectral concentration of N-acetylglutamine in the follow-up samples of those with Definite SIBO. If this were found to be the case, there would be a strong case for continued research into this metabolite.

Following this, there would be two further steps in the metabolomics biomarker discovery process: (1) study validation and (2) cohort validation (Dunn et al. 2010). The 'study validation' would effectively involve another case-control pilot study, with participant numbers still quite small i.e. in the range of 20-100s for each class. However, a more robust study design would be required, whereby the patients and controls were more appropriately selected. This would mean having three patient groups in total, with the latter two groups acting as controls: (1) patients with GI symptoms and Definite SIBO; (2) age-, gender-, race- and BMI-matched patients with GI symptoms but No SIBO and; (3) age-, gender-, race- and BMI-matched healthy individuals with no GI symptoms and No SIBO. This process of carefully matching comparison groups would mean that the validated test would be appropriate for use in clinical practice. This is important because all individuals presenting to the gastroenterologist will have GI symptoms, and the gastroenterologist will need the diagnostic test to determine whether they have SIBO or not. At this point, one may also wish to undertake an inter-laboratory repeatability study that uses replicate specimens from the same cases and controls, but the experiment is performed in a

different laboratory using a different instrument (potentially the same manufacturer) and a different observer (Xia et al. 2012).

Assuming, the study validation supported the discovery of the original pilot study and there was low variability due to independent laboratory practices, the final cohort validation study could then be undertaken. This would be a medium to large-scale epidemiological study (i.e. 1,000's) of the complete at-risk population. Large numbers would be required to take account of the substantial diversity observed in physiology, metabolic status, diet and lifestyle in the general population. A large sample size will also serve to boost the power of statistical analysis, so that subtle differences within the subject cohort would be detected, thereby reducing the probability of false discovery. This final study would define the true utility of the *'discovered'* biomarker in the target population. Only then could one be sure that the biomarker was reproducible, with optimal sensitivity and specificity.

5.6.2.1 Strengths and Limitations of Metabolomics Methods and Results

The conditions under which the urine samples were stored and prepared for this study needs consideration, as storage conditions are likely to have a major impact on the content of the multivariate data set presented (Dunn et al. 2011). A good understanding of the analytical variation is critically important in any metabolic profiling study as it allows the separation of artifactual and analytical variation from the biological variations of interest (i.e. the disease). Therefore, in recent years, urine sample preparation guidelines for metabolomics studies have been proposed (Lauridsen et al. 2007; Maher et al. 2007). These guidelines will now be discussed in the context of the current study.

Maher et al. investigated the effect of long-term storage of urine at different temperatures (-80°C, -40°C, -20°C, 4°C and room temperature) with the aim of assessing the consequences of delayed freezing (2007). Samples were analysed the day after collection, at 1 month and at 3 months. From PCA, it was evident that the major source of variation was time-dependent changes associated with 1 month and 3 month room-temperature storage. However, apart from

this cause of variation, the remainder of the data (i.e. for -80°C, -40°C, -20°C and 4°C) was clustered together, thus showing little systematic variation. This suggests that the storage of the urine samples in the current study (at -20°C) was acceptable. This is supported by the recommendations of Lauridsen et al., who proposed that human urine samples should be stored at or below -25°C, as no changes in the ^{1}H NMR fingerprints were observed at this temperature for 26 weeks (2007). However, in the current study, samples were generally not frozen ≤ 2 hours after collection (i.e. they may have been at room temperature and refrigerated for up to 36 hours), as was the case with Maher and Lauridsen's samples. Also, some samples were analysed < 26 weeks after collection but others were not analysed until 18 months after collection. There has been no research to date focused on measuring sample stability when frozen for > 26 weeks and therefore, one cannot be certain of the trustworthiness of the data produced for the samples with longer freezing periods.

Previous experiments on human urine indicate that preservatives (sodium azide or sodium fluoride) extend the stability of urine at 4°C (Lauridsen et al. 2007). In the current study, a preservative was not used, but ideally should have been, as it may have alleviated any microbial contamination caused by holding samples at room or refrigeration temperatures for up to 36 hours. In addition, the collection of urine samples on site (rather than at home) would have resulted in a shorter collection to freezing time. However, such a measure to ensure consistency of sample collection may have negatively affected the number of samples obtained. Of note, participants' medication, dietary (including alcohol) intake and physical activity level during the 24-hour period prior to sample collection can influence the metabolomic spectrum (Holmes et al. 1994; Zuppi et al. 1998; Maher et al. 2007). Ideally these variables should have been recorded to reduce the chance of introducing systematic bias into the data.

At the ^{1}H NMR analysis stage, there were some study strengths. Given the relatively low numbers of patients in the study, all samples were analysed in a single analytical batch. This removed the possibility of batch effects i.e. technical sources of variation that have been added to the samples during handling. Also, the sample preparation order and injection order (i.e.

injection into NMR tubes) was randomized so that controls and test samples were run on the spectrometer in a random order i.e. avoiding run-order bias.

In addition to these sample handling and storage issues, metabolomics studies have been criticised for being *'fishing expeditions'* i.e. one is likely to discover biomarkers that are randomly correlated to the effect of interest. In the current study, the t-testing significance value was set at $p< 0.02$. This is not ideal even when just one t-test is performed, but as many tests were performed, this hugely increases the chance of false discoveries i.e. random findings. Although, the Benjamini-Hochberg procedure was used to correct for multiple testing, the validity of this methods in *'omic'* type studies has been questioned (Dunn et al. 2010). Also, supervised classification is an inherently biased technique: Adaptive Intelligent Binning was used in the current study, with multiple comparisons necessary to complete the binning procedure. Normally, Bonferroni corrections would be made to correct for familywise error, but this was not possible because the variables (bins) were not independent of one another. As a result, there is a risk that the metabolite identified as significant was the result of a false discovery.

Evidently, a drawback of all metabolomics research is the need to use complex data-interpretation techniques and combinations of analytical methods (Nicholson & Lindon 2008). For example, when modeling data, it is possible to obtain a model that provides a good fit to the data, as was the case with the current study. However, if the model was over-fitted because of the modeling of noise, then the predictive ability will be negatively affected, as any new data set will have a different noise component (Lindon et al. 2005). In the current study, although the data was visualised to assess for the presence of noise, ideally other more sophisticated methods would have been employed (Lindon et al. 2005). For example, internal cross-validation is a method that ensures that the model size can be determined directly from the data. This method can differentiate the regularities in the data from noise and give a realistic estimate of the predictive capability of the model/metabolite (i.e. prevent over-fitting) (Lindon et al. 2005).

5.7 Conclusions

Small intestinal bacterial overgrowth is prevalent in symptomatic patients following oncological treatment: one in five of those tested were confirmed to have the condition. The GI burden of SIBO is great, with more upper- and lower-GI symptoms accompanying it than have previously been described. The current study was unable to define risk factors for the development of SIBO, but univariate results suggest which variables should be considered in future modeling studies (selenium status and previous radiotherapy/brachytherapy treatment). There exists no ideal test to detect the presence of SIBO, with inconsistencies between methods demonstrated here. This has been the first attempt to investigate the potential of metabolomics in SIBO by comparing the urine metabolic fingerprints in those with and without SIBO. Although, there is not yet any conclusive indication that metabolomics can separate those with SIBO, it remains an exciting avenue to explore in the hope of finding a more superior test for the diagnosis of this troublesome condition.

Chapter 6

Final Discussion and Conclusions

6.1 Summary of Key Findings

The assessment of the development and persistence of GI symptoms and malnutrition in patients with OG cancer were two objectives of this thesis. The symptom burden was high for the first year following diagnosis: for the majority, symptoms either persisted without any improvement or developed, during this time (Chapter 3). At one year, seven in ten patients reported having at least one GI symptom that bothered them *'quite a bit'* or *'a great deal'*. Similarly, malnutrition was a significant feature in this cancer cohort, with six in ten patients either becoming malnourished or remaining malnourished at one year. Additionally, high GI symptom burden tended to be associated with poorer nutritional status and low symptom burden tended to be associated with better nutritional status. Considering the high prevalence of malnutrition in OG cancer patients, there is a need to validate a nutritional screening tool for use in this group. A validation study was undertaken in this thesis to establish the sensitivity and specificity of MUST against an accepted standard (Chapter 4). The tool was found to be insufficiently sensitive and thus, failed to identify patients at risk of malnutrition. Therefore, it does not appear to be suitable for use in the OG oncology setting.

Interestingly, the incidence of SIBO in this OG cancer cohort was also high at 77%. This indicates, for the first time, that disease processes and/or oncological treatments may lead to the development of SIBO. It is reasonable to hypothesise that SIBO may be contributing to the chronic GI symptoms and poor nutritional status experienced by OG cancer patients (Chapter 3). This thesis has also established that SIBO is not unique to the OG cancer setting, but rather it is a common finding in cancer patients, in general. One in every five patients presenting to the gastroenterologist with GI symptoms following treatment for (any) cancer was found to have SIBO (Chapter 5). The range of symptoms reported by these patients was extensive: flatulence, faecal urgency, loose stools, abdominal grumbling, incomplete evacuation, steatorrhoea and faecal incontinence were the most common. Some of these symptoms, namely faecal urgency, abdominal grumbling, incomplete evacuation and faecal incontinence, as well as some others, including belching and early satiety, have never been considered relevant to SIBO before. As

such, this research has exposed SIBO as being a more troublesome condition that was previously believed.

There did not appear to any defining traits of SIBO, nor strong predictors for its development, which further stresses the need for a sensitive and specific test to diagnose it. The analysis of metabolites in urine using metabolomics was trialled in this thesis, in an attempt to ascertain whether this approach could eventually lead to a new diagnostic test for SIBO. The technology showed some ability to classify those with and without SIBO based on N-acethylglutamine, thus demonstrating its potential in this setting.

6.2 Management of Gastrointestinal Symptoms and Malnutrition in Oesophagogastric Cancer

The persistence of moderate-severe GI symptoms during the first year following OG cancer diagnosis was established in this thesis. Considering these patients had ongoing troublesome symptoms, it is evident that the current symptom detection and treatment processes are not optimal. This raises two questions: (1) What is the best way to detect GI symptoms? and (2) Who are the best clinicians to manage GI symptoms?

Focusing on Question 1 firstly (What is the best way to detect GI symptoms?). When patients are first diagnosed with OG cancer, they undergo extensive staging investigations, where the central emphasis is on treatment planning. As such, GI function tends to become a peripheral factor in their overall management. However, this thesis has demonstrated that GI symptoms are already prevalent at this stage. Therefore, those initial out-patient appointments should represent a key moment to undertake a comprehensive GI symptom assessment. During and after the completion of treatment, the emphasis tends to shift, with an increased awareness of the acute and chronic GI side-effects of cancer therapies. Moreover, the recently published London Cancer Alliance (LCA) guidelines include a section dedicated to identifying post-treatment symptoms during follow-up (Oesophageal and Gastric Cancer Clinical Guidelines, London Cancer Alliance 2014). They highlight the need for ongoing assessment of GI function

and recommend that follow-up (including GI symptom assessment) should occur as follows: (a) two weeks post-discharge, (b) regularly in the first year- the frequency should be determined by the post-treatment symptoms, (c) six monthly for two years and, (d) annually until five years in total. If implemented, this guideline would ensure that GI symptom assessment became a core component of all follow-ups, leading to improved symptom detection.

Focusing on Question 2 now (Who are the best clinicians to manage GI symptoms?). The LCA guidelines suggest that the approach to follow-up should be uniform irrespective of discipline. Therefore, any member of the MDT could undertake the GI symptom assessment and then communicate the outcome to the other members, thus avoiding duplication. This approach would ensure that all clinicians are mindful of potential GI symptoms. However, there is no guidance on which clinicians are best equipped to manage complex symptoms once they've been detected. As the type of treatment modality/modalities received will determine whether a patient is followed-up by a surgeon and/or clinical oncologist, and assuming these clinicians would manage symptoms differently, this means there is no standard approach for managing symptoms. An ongoing prospective, observational cohort study at RM (The FOCCUS Study: Focusing on Cancers Chemotherapys' Untreated Symptoms) will address this issue. Aims of the study include (a) defining the competencies required of nursing personnel to use a monitoring, investigation and treatment algorithm and (b) developing simple protocols for clinicians to use to assist with symptom management. The results of this novel study will provide guidance on the most appropriate method for managing and treating GI symptoms in OG cancer patients, which could greatly reduce the prevalence and persistence of troublesome symptoms.

With regard to nutritional status, this thesis has demonstrated that malnutrition is not being effectively detected and treated in all OG cancer patients. Therefore, current detection and treatment processes do not appear to be optimal. There is a need for change. The LCA guidelines also include a section on nutrition (2014). The alliance recommends that all OG cancer patients should have a nutritional assessment carried out at presentation, which conforms to national and international best practice. In addition, depending on which treatment

modalities apply, they advise (a) ongoing nutritional assessment during chemotherapy, (b) weekly nutritional assessment during chemoradiotherapy, (c) ongoing follow-up after the completion of chemotherapy/chemoradiation and, (d) long-term follow-up in surgical patients.

Thus, the guidelines suggest that the best way to improve nutritional status is to provide sufficient dietetic resources to meet the needs of patients. In practice this will prove difficult to achieve, as dietetic resources are limited. Even the systematic introduction of one of these targets would have major cost implications caused by an increased number of referrals to dietetic departments and an increased use of nutritional support. But while this may cause short-term financial losses, it is likely to be counteracted by long-term gains produced by clinical benefits. This is because dietetic counselling and intervention have been shown to improve energy intake, weight gain, functional status and QoL in cancer patients (Persson et al. 2002; Ravasco, Monteiro-Grillo, Vidal & Camilo 2005a; Kim et al. 2014; Isenring et al. 2004; Ravasco, Monteiro-Grillo, Vidal & Camilo 2005b). These clinical benefits may also lead to reduced complications and lengths of hospital stay.

Nonetheless, a great deal of collaborative planning is essential before the LCA nutritional guidelines can be incorporated into routine clinical practice. While the cost and feasibility issues are being considered, it is paramount that nutritional screening processes are fully embedded in the standard care of OG cancer patients as per National Institute for Health and Clinical Excellence guidance: all out-patients should be screened at their first clinic appointment and when there is clinical concern thereafter; all in-patients should be screened on admission and weekly thereafter (National Institute for Health and Care Excellence 2006). In this way, only those at risk of malnutrition would undergo a full nutritional assessment (rather than those not at risk), thus saving dietetic resources. Importantly, this can only be achieved if the screening tool is highly sensitive and specific, which emphasises the significance of choosing a suitable tool for use in OG cancer patients. This thesis has established that MUST is not a suitable tool for use in this patient group because of its low sensitivity. Its use would cause some of those OG cancer patients at greatest risk of malnutrition to remain undetected, making the screening

process ineffective. Considering MUST is the most commonly used nutritional screening tool in the UK, this finding has significant implications for the management of this high-risk patient group.

Another matter of great importance in the management of OG cancer patients is that a patient-centred approach is ensured at all times. Historically, clinicians tend to follow a prescriptive approach resulting in patients being told what to do and when to do it. Therefore, patients are not taught to be pro-active if problems arise e.g. persistence of symptoms, weight loss or eating difficulties. However, if all patients were given information explaining what issues were abnormal, and they were encouraged to self-monitor, then this would empower them to know when to seek help from the appropriate professional(s), rather than waiting for the next follow-up appointment. This collaborative approach is also endorsed by the LCA and they provide a noteworthy suggestion in their clinical guidelines: '*Information on anticipated or possible consequences of cancer treatment and what to do if they occur should be routinely provided to all patients. This should be done from the time of discussion of treatment onwards, with the information reiterated during the end of treatment consultation'* (London Cancer Alliance 2014). If this were realised, it would allow patients to seek early intervention and avoid having to endure ongoing unresolved GI or nutritional issues.

6.2.1 Future Research Considerations

This thesis demonstrated that symptom burden showed an association with nutritional status in OG cancer patients, whereby the presence of symptoms tended to be associated with poorer nutritional status and *vice versa*. As such, it seems reasonable to hypothesise that the effective treatment of GI symptoms that are negatively impacting on dietary intake would improve nutritional status. This would be an interesting focus for future research. In addition, as MUST was found to be inappropriate for use in OG cancer patients, and the relationship between GI symptoms and nutritional status was established in this cohort, one can postulate that a screening tool which incorporates symptom assessment may perform better than MUST. The Royal Marsden Nutrition Screening Tool, which assesses nutrition-impact symptoms, has

recently been validated (against PG-SGA) in an mixed in-patient cancer cohort (Shaw et al. 2014). Therefore, the next, most logical step would be to establish its performance in patients with OG cancer, who may be in- or out-patients.

This thesis has also provided evidence to suggest that SIBO may be contributing to the chronic GI symptoms and malnutrition experienced by OG cancer patients. Due to the low number of patients undergoing testing for SIBO in this part of the study, further prospective research is needed to establish such relationships. If the cause for their persistent symptoms and malnutrition was found to be related to SIBO, this would have significant implications for their management during and after treatment.

6.3 Feasibility of Metabolomics in Clinical Practice for Small Intestinal Bacterial Overgrowth Detection

In the past 10-15 years, continuous advances in the application of NMR to the diagnosis and characterisation of diseases have made metabolomics a useful clinical option, rather than just a scientific curiosity. Metabolomics technology has the potential to provide fast, accurate, non-invasive diagnosis of complex medical problems. The successful application of urine metabolomics as a non-invasive strategy could circumvent and/or complement the established invasive and time-consuming clinical procedures used for SIBO detection. However, the challenges and needs in disease metabolomics require careful consideration, namely in relation to study design, sample collection and quality, data quality assurance, reliable means of data analysis and model validation and, finally, confirmation of metabolite biomarkers (as discussed in Section 5.6.2). All of these aspects would need to be perfected before one could envisage the application of NMR in SIBO detection, as has already been achieved to allow discrimination of the inflammatory bowel diseases from each other and from healthy individuals (Williams et al 2009). Assuming these aspects were achieved for SIBO, further practical considerations of NMR would need to be considered before a link between the laboratory and clinic could be established.

Firstly, the cost of NMR deserves attention. Analytical platforms are expensive (£100,000 per spectrometer) and require a facility manager to oversee the day-to-day running of the technology (Dunn et al. 2011). As a result, the majority of spectrometers are currently located in industrial or university settings rather than clinical settings. Therefore, until a time comes when spectrometers are routinely found in hospitals, samples will continue to require freezing and transportation at a later date and of course this introduces bias, as discussed in Section 5.6.2.1. On a positive note, once the equipment is in place, there are actually few costs associated with running the NMR experiments: for a basic 1D experiment, the cost is approximately £10 per sample. For structural elucidation experiments (i.e. 2D analysis), the cost rises but the total cost compared with other *'omic'* platforms (e.g. transcriptomics or proteomics) is still much lower (Dunn et al. 2011). If a biomarker was found to be robust enough to supersede an established test for SIBO, it is possible that the cost of metabolomics would be no greater than the combined (average) cost of a GHMBT (£125) and OGD with jejunal aspiration (£490) to the NHS (Gov UK 2014; University College London Hospitals 2013).

Secondly, one must consider how quickly clinically relevant results can be obtained from the metabolomics platform. Encouragingly, sample preparation is composed of a limited number of processes, and many steps (e.g. liquid handling and extraction) can be automated. Also, the high throughput nature of metabolic profiling means that each sample can be analysed (by the spectrometer) typically in less than 30 minutes. Despite the efficiency in obtaining the spectra, the complex pre-processing and data interpretation techniques require much more time. It may take days or even weeks before any meaningful results can be relayed to the patient. However, if the technology can be proven superior to the direct aspiration and breath testing methods for detecting SIBO, then it may be worth the wait.

Thirdly, as metabolomics is a novel discipline encompassing comprehensive metabolite evaluation, pattern recognition, and statistical analyses, the skills required are typically the remit of the trained scientist, rather than the clinician. Consequently, there exists a perception that metabolomics technology is complicated, unreliable and inaccessible to the clinical world.

Changing this perception may be challenging but it is essential that clinicians become more familiar with metabolomics data, as a good working knowledge will be required to make optimal use of this promising technology. If this were achieved, the time period between sample collection and obtaining clinical meaningful results would be reduced. Accordingly, prompt intervention could be commenced, as required, without the need for an intermediary in the pathway. This need to expand systems biology from the borders of the basic biology and mathematical communities into the clinical world has been highlighted in a European Commission report (Directorate of Health 2010). Also, the Medical Research Council in the UK is supporting *'discipline hopping'* through fellowship schemes that enable scientists and clinicians to do research in fields different from their own (Medical Research Council 2014). Metabolomics technology is a discipline that is still in its infancy and multidisciplinary efforts are essential to ensure the gap between the laboratory and the clinic is bridged.

6.3.1 Future Research Considerations

This thesis has demonstrated the potential of urine metabolomics technology in the pursuit of a new diagnostic test for SIBO, advocating future research in this area. Urine was the sample of choice in Chapter 5 because, to date, this biofluid has received the greatest attention with regard to metabolite elucidation as compared with duodenal aspirate or faecal water samples. However, one can envisage that the metabolic profiles of the duodenal aspirate and faecal water samples (also collected in this cancer cohort) could hold even more potential, as they are the direct product of gut microbiome-host co-metabolism. As such, significant metabolite signals may be more easily detected from these samples than from urine. Therefore, in the coming months, as well as assessing the metabolic profiles of the follow-up urine samples (i.e. post-antibiotic treatment) from the SIBO Study, the duodenal aspirate and faecal water samples will also be analysed and interpreted using metabolomics technology. Ultimately, a combination of biomarkers from two or three biofluids could prove superior to the currently available diagnostic tests for SIBO.

6.4 Concluding statement

Cancer is a complex disease resulting in multifaceted effects. The management of localised OG cancer is aggressive and as such, the GI and nutritional consequences are pronounced during the first year following presentation. Many patients endure unrelenting symptoms while in a state of malnutrition and the association between these variables is strongest at diagnosis. In OG cancer and cancer in general, SIBO is likely to be contributing to the GI symptoms experienced following treatment. Despite SIBO's relevance across the field of gastroenterology, there is still no appropriate method to diagnose it. Urine metabolomics has, however, shown great potential in its ability to detect patients with SIBO, making it an exciting prospect in the quest for a simple, accurate and objective test for SIBO.

In summary, this thesis has developed and tested the following hypotheses:

1. The hypothesis that disease processes and/or radical treatment result in the persistence or development of moderate-severe GI symptoms at 12 months in OG cancer patients was accepted (Chapter 3).
2. The hypothesis that disease processes and/or radical treatment result in the persistence or development of malnutrition at 12 months in OG cancer patients was accepted (Chapter 3).
3. The hypothesis that there is a positive association between GI symptom scores (higher score equals worse GI symptoms) and nutritional status scores (higher score equals worse nutritional status) at diagnosis, during the acute phase of management and chronically in OG cancer patients was accepted (Chapter 3).
4. The hypothesis that MUST has an acceptable sensitivity and specificity (≥ 70% for both) in the OG oncology setting, by comparison with PG-SGA was not accepted (Chapter 4).
5. The hypothesis that qualitative and quantitative analyses of metabolites in urine will indicate the presence or absence of SIBO in patients treated for cancer was not accepted (Chapter 5).

Chapter 7

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Chapter 8

Appendices

8.1 TNM Classification of Malignant Tumours; 7th Edition

TNM Clinical Classification: Oesophagus and Gastro-oesophageal Junction

(Sobin et al. 2009)

T: Primary Tumour

Tx Tumour cannot be assessed

T0 No evidence of tumour

Tis High-grade dysplasia

T1 Tumour invades lamina propria, muscularis mucosae or submucosa

T2 Tumour invades into but not beyond the muscularis propria

T3 Tumour invades the paraesophageal tissue, but does not invade adjacent structures

T4 Tumour invades adjacent structures

T4a Resectable tumour invades adjacent structures, such as pleura, pericardium, diaphragm

T4b Unresectable tumour invades other adjacent structures, such as aorta, vertebral body, trachea

N: Regional Lymph Nodes

Any perioesophageal lymph node from cervical lymph nodes to celiac node

Nx Lymph nodes cannot be assessed

N0 No lymph node metastasis

N1 1-2 positive lymph nodes

N2 3-6 positive lymph nodes

N3 >6 positive lymph nodes

M: Distant Metastasis

Mx Distant metastasis cannot be assessed

M0 No distant metastasis

M1 Distant metastasis

Note: Gastro-oesophageal junction includes cancers with an epicenter in the distal thoracic esophagus, GOJ, or within the proximal 5 cm of the stomach (cardia) that extend into the GOJ or oesophagus and are stage grouped similar to adenocarcinoma of the oesophagus.

TNM Clinical Classification: Stomach (Washington 2010)

T: Primary Tumour

Tx Tumour cannot be assessed

T0 No evidence of tumour

Tis Carcinoma in situ

T1 Tumour invades lamina propria, muscularis mucosae or submucosa.

T2 Tumour invades muscularis propria

T3 Tumour invades subserosa connective tissue without invasion of visceral peritoneum or adjacent structures

T4 Tumour invades serosa (visceral peritoneum) or adjacent structures

T4a: Tumour invades serosa but not adjacent structures

T4b: Tumour invades adjacent structures such as spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine, and retroperitoneum

N: Regional Lymph Nodes

Nx Lymph nodes cannot be assessed

N0 No lymph node metastasis

N1 1-2 positive lymph nodes

N2 3-6 positive lymph nodes

N3 >6 positive lymph nodes

M: Distant Metastasis

Mx Distant metastasis cannot be assessed

M0 No distant metastasis

M1 Distant metastasis

8.2 Academy/A.S.P.E.N. Clinical Characteristics that the Clinician Can Obtain and Document to Support a Diagnosis of Malnutrition

Reference: White et al. 2012

Clinical Characteristic	Malnutrition in the Context of Acute Illness or Injury		Malnutrition in the Context of Chronic Illness		Malnutrition in the Context of Social or Environmental Circumstances	
	Nonsevere (Moderate) Malnutrition	**Severe Malnutrition**	**Nonsevere (Moderate) Malnutrition**	**Severe Malnutrition**	**Nonsevere (Moderate) Malnutrition**	**Severe Malnutrition**
(1) Energy intake Malnutrition is the result of inadequate food and nutrient intake or assimilation; thus, recent intake compared with estimated requirements is a primary criterion defining malnutrition. The clinician may obtain or review the food and nutrition history, estimate optimum energy needs, compare them with estimates of energy consumed and report inadequate intake as a percentage of estimated energy requirements over time.	<75% of estimated energy requirement for >7 days	≤50% of estimated energy requirement for ≥5 days	<75% of estimated energy requirement for ≥1 mo	≤75% of estimated energy requirement for ≥1 mo	<75% of estimated energy requirement for ≥3 mo	≤50% of estimated energy requirement for ≥1 mo
(2) Interpretation of weight loss The clinician may evaluate weight in light of other clinical findings, including the presence of under- or over-hydration. The clinician may assess weight change over time reported as a percentage of weight lost from baseline.	1-2% in 1 wk 5% in 1 mo 7.5% in 3 mo	>2% in 1 wk >5% in 1 mo >7.5 in 3 mo	5% in 1 mo 7.5% in 3 mo 10% in 6 mo 20% in 1y	>5% in 1 mo >7.5% in 3 mo >10% in 6 mo >20% in 1y	5% in 1 mo 7.5% in 3 mo 10% in 6 mo 20% in 1y	>5% in 1 mo >7.5% in 3 mo >10% in 6 mo >20% in 1y
Physical Findings Malnutrition typically results in changes to the physical exam. The clinician may perform a physical exam and document any one of the physical exam findings below as an indicator of malnutrition.						
(3) Body fat Loss of subcutaneous fat (eg, orbital, triceps)	Mild	Moderate	Mild	Severe	Mild	Severe

Clinical Characteristic	Malnutrition in the Context of Acute Illness or Injury		Malnutrition in the Context of Chronic Illness		Malnutrition in the Context of Social or Environmental Circumstances	
	Nonsevere (Moderate) Malnutrition	Severe Malnutrition	Nonsevere (Moderate) Malnutrition	Severe Malnutrition	Nonsevere (Moderate) Malnutrition	Severe Malnutrition
(4) Muscle mass Muscle loss (e.g. wasting of the temples [temporalis muscle], clavicles [pectoralis and deltoids], shoulders [deltoids], interosseous muscles, scapula [latissimus dorsi, trapezious, deltoids], thigh [quadriceps], and calf [gastrocnemius])	Mild	Moderate	Mild	Severe	Mild	Severe
(5) Fluid accumulation The clinician may evaluate generalized or localized fluid accumulation evident on exam (extremities, vulvar/scrotal edema, or ascites). Weight loss is often masked by generalized fluid retention (edema), and weight gain may be observed.	Mild	Moderate to severe	Mild	Severe	Mild	Severe
(6) Reduced grip strength Consult normative standards supplied by the manufacturer of the measurement device	NA	Measurably reduced	NA	Measurably reduced	NA	Measurably reduced

A minimum of 2 of the 6 characteristics above is recommended for diagnosis of either severe or nonsevere malnutrition. NA, not applicable.

Notes:
Height and weight should be measured rather than estimated to determine body mass index (BMI).
Usual weight should be obtained to determine the percentage and to interpret the significance of weight loss.
Basic indicators of nutrition status such as body weight, weight change, and appetite may substantively improve with refeeding in the absence of inflammation.
Refeeding and/or nutrition support may stabilize but not significantly improve nutrition parameters in the presence of inflammation.
The National Center for Health Statistics defines *chronic* as a disease/condition lasting 3 months or longer.
Serum proteins such as serum albumin and prealbumin are not included as defining characteristics of malnutrition because recent evidence analysis shows that serum levels of these proteins do not change in response to changes in nutrient intake.

8.3 Patient Generated Subjective Global Assessment

Reference: Ottery 2000

Scored Patient-Generated Subjective Global Assessment (PG-SGA)

Patient ID Information

History (Boxes 1-4 are designed to be completed by the patient.)

1. Weight *(See Worksheet 1)*

In summary of my current and recent weight:

I currently weigh about _____ pounds
I am about _____ feet _____ tall

One month ago I weighed about _____ pounds
Six months ago I weighed about _____ pounds

During the past two weeks my weight has:
☐ decreased (1) ☐ not changed (0) ☐ increased (0)

Box 1 ☐

2. Food Intake: As compared to my normal intake, I would rate my food intake during the past month as:

☐ unchanged (0)
☐ more than usual (0)
☐ less than usual (1)

I am now taking:

☐ *normal food* but less than normal amount (1)
☐ little solid food (2)
☐ only liquids (3)
☐ only nutritonal supplements (3)
☐ very little of anything (4)
☐ only tube feedings or only nutrition by vein (0)

Box 2 ☐

3. Symptoms: I have had the following problems that have kept me from eating enough during the past two weeks (check all that apply):

☐ no problems eating (0)
☐ no appetite, just did not feel like eating (3)
☐ nausea (1)
☐ constipation (1)
☐ mouth sores (2)
☐ things taste funny or have no taste (1)
☐ problems swallowing (2)
☐ pain; where? (3)_____
☐ other** (1) _____
☐ vomiting (3)
☐ diarrhea (3)
☐ dry mouth (1)
☐ smells bother me (1)
☐ feel full quickly (1)
☐ fatigue (1)

** Examples: depression, money, or dental problems

Box 3 ☐

4. Activities and Function: Over the past month, I would generally rate my activity as:

☐ normal with no limitations (0)
☐ not my normal self, but able to be up and about with fairly normal activities (1)
☐ not feeling up to most things, but in bed or chair less than half the day (2)
☐ able to do little activity and spend most of the day in bed or chair (3)
☐ pretty much bedridden, rarely out of bed (3)

Box 4 ☐

Additive Score of the Boxes 1-4 ☐ A

The remainder of this form will be completed by your doctor, nurse, dietitian, or therapist. Thank you.

Scored Patient-Generated Subjective Global Assessment (PG-SGA)

Worksheet 1 - Scoring Weight (Wt) Loss

To determine score, use 1 month weight data if available. Use 6 month data only if there is no 1 month weight data. Use points below to score weight change and add one extra point if patient has lost weight during the past 2

Wt loss in 1 month	Points	Wt loss in 6 months
10% or greater	4	20% or greater
5-9.9%	3	10 -19.9%
3-4.9%	2	6 - 9.9%
2-2.9%	1	2 - 5.9%
0-1.9%	0	0 - 1.9%

Numerical score from Worksheet 1 ☐

Additive Score of the Boxes 1-4 (See Side 1) ☐ A

5. Worksheet 2 - Disease and its relation to nutritional requirements

All relevant diagnoses (specify) _____

One point each:

☐ Cancer ☐ AIDS ☐ Pulmonary or cardiac cachexia ☐ Presence of decubitus, open wound, or fistula
☐ Presence of trauma ☐ Age greater than 65 years ☐ Chronic renal insufficiency

Numerical score from Worksheet 2 ☐ B

6. Work Sheet 3 - Metabolic Demand

Score for metabolic stress is determined by a number of variables known to increase protein & calorie needs. The score is additive so that a patient who has a fever of > 102 degrees (3 points) and is on 10 mg of prednisone chronically (2 points) would have an additive score for this section of 5 points.

Stress	none (0)	low (1)	moderate (2)	high (3)
Fever	no fever	>99 and <101	≥101 and <102	≥102
Fever duration	no fever	<72 hrs	72 hrs	> 72 hrs
Corticosteroids	no corticosteroids	low dose (<10mg prednisone equivalents/day)	moderate dose (≥10 and <30mg prednisone equivalents/day)	high dose steroid (≥30mg prednisone equivalents/day)

Numerical score from Worksheet 3 ☐ C

7. Worksheet 4 - Physical Exam

Physical exam includes a subjective evaluation of 3 aspects of body composition: fat, muscle, & fluid status. Since this is subjective, each aspect of the exam is rated for degree of deficit. Muscle deficit impacts point score more than fat deficit. Definition of categories: 0 = no deficit, 1+ = mild deficit, 2+ = moderate 3+ = severe

Muscle Status:				
temples (temporalis muscle)	0	1+	2+	3+
clavicles (pectoralis & deltoids)	0	1+	2+	3+
shoulders (deltoids)	0	1+	2+	3+
interosseous muscles	0	1+	2+	3+
Scapula (latissimus dorsi, trapezius, deltoids)	0	1+	2+	3+
thigh (quadriceps)	0	1+	2+	3+
calf (gastrocnemius)	0	1+	2+	3+
Global muscle status rating	**0**	**1+**	**2+**	**3+**
Fat Stores:				
orbital fat pads	0	1+	2+	3+
triceps skin fold	0	1+	2+	3+
fat overlying lower ribs	0	1+	2+	3+
Global fat deficit rating	**0**	**1+**	**2+**	**3+**

Fluid Status:				
ankle edema	0	1+	2+	3+
sacral edema	0	1+	2+	3+
ascites	0	1+	2+	3+
Global fluid status rating	**0**	**1+**	**2+**	**3+**

Numerical score from Worksheet 4 ☐ D

Total PG-SGA score ☐

(Total numerical score of A+B+C+D above)

(See triage recommendations below)

Global PG-SGA rating (A, B, or C) = ☐

Clinician Signature _____ RD RN PA MD DO Other ___ Date _____

Worksheet 5 - PG-SGA Global Assessment Categories

	Stage A	Stage B	Stage C
Category	Well nourished	Moderately malnourished	Severely malnourished
Weight	No wt loss **OR** Recent wt gain	≤ 5% wt loss in 1 month (or 10% in 6 mos) **OR** Progressive wt loss	> 5% wt loss in 1 month (or >10% in 6 mos) **OR** Progressive wt loss
Nutrient intake	No deficit **OR** Significant recent improvement	Definite decrease in intake	Severe deficit in intake
Nutrition Impact Symptoms	None **OR** Singificant recent improvement allowing adequate intake	Present of nutrition impact symptoms (PG-SGA Box 3)	Present of nutrition impact symptoms (PG-SGA Box 3)
Functioning	No deficit **OR** Recent improvement	Moderate functional deficit **OR** Recent deterioration	Severe functional deficit **OR** recent significant deterioration
Physical Exam	No deficit **OR** Chronic deficint but recent improvement	Evidence of mild to moderate loss of muscle mass / SQ fat / muscle tone on palpation	Obvious signs of malnutrition (eg, severe loss muscle, SQ tissue, possible edema)

Nutritional Triage Recommendations: Additive score is used to define specific nutritional interventions including patient & family education, symptom management including pharmacologic intervention, and appropriate nutrient intervention (food, nutritional supplements, enteral, or parenteral triage).

First line nutrition intervention includes optimal symptom management.

Triage based on PG-SGA point score

0-1	No intervention required at this time. Re-assessment on routine and regular basis during treatment.
2-3	Patient & family education by dietitian, nurse, or other clinician with pharmacologic intervention as indicated by symptom survey (Box 3) and lab values as appropriate.
4-8	Requires intervention by dietitian, in conjunction with nurse or physician as indicated by symptoms (Box 3).
≥ 9	Indicates a critical need for improved symptom management and/or nutrient intervention options.

8.4 CCR 3703 Modified Gastrointestinal Symptom Rating Scale

Patient Information: to be completed by the ***dietitian***   **Step 1, Step 2 and Step 3**: to be completed by the ***study participant***

Patient Information (*to be completed by the dietitian*)   Date: _____   Study number: _____   Is it a baseline measurement? ☐ yes ☐ no   Is it a follow-up measurement? ☐ yes ☐ no   Tick the follow-up month as appropriate: ☐ 3 ☐ 12

STEP 1 (to be completed by the ***study participant***)

Please take a few moments to think about any **bowel symptoms** you have had over the past 4 weeks. Please indicate whether you have had bowel symptoms during this time and how severe they were using the scale below.

	In the past month, I would rate my overall bowel symptoms as: (please tick one)
1. **None:** I didn't have any symptoms	
2. **Mild:** I had a/some symptoms but they didn't bother me	
3. **Moderate:** I had symptoms often, they bothered me quite a bit	
4. **Severe:** I had a lot of symptoms, they bothered me a great deal	

STEP 2 (to be completed by the ***study participant***)

Please rate your bowel symptoms over the past 4 weeks by placing a tick in the box that best describes your symptoms. (*Please tick none if you do not have this symptom.)*

	I didn't have this symptom	**I had it a bit but it didn't bother me much**	**I had it often, it bothered me quite a bit**	**I had it a lot, bothered me a great deal**
	None	**Mild**	**Moderate**	**Severe**
1. Abdominal pain / discomfort (any kind of pain in your belly)	☐	☐	☐	☐
2. Heartburn (burning / discomfort behind breastbone)	☐	☐	☐	☐
3. Acid reflux / acid regurgitation (flow of sour fluid into your mouth)	☐	☐	☐	☐
4. Nausea (feeling of wanting to be sick)	☐	☐	☐	☐
5. Abdominal rumbling / gurgling (bubbling or noise in the stomach)	☐	☐	☐	☐
6. Abdominal bloating / distension (swelling in the stomach or belly)	☐	☐	☐	☐
7. Belching / burping (bringing up gas through the mouth)	☐	☐	☐	☐
8. Flatulence / passing wind (release of gas from the bottom)	☐	☐	☐	☐
9. Constipation (stools are infrequent / difficult to pass)	☐	☐	☐	☐
10. Diarrhoea (loose or watery stools)	☐	☐	☐	☐
11. Loose stools (mushy or watery stools)	☐	☐	☐	☐
12. Hard stools (lumpy or dry stools)	☐	☐	☐	☐
13. Urgency to open bowels (rushing to do a poo)	☐	☐	☐	☐
14. Incomplete evacuation (feeling of something left behind after	☐	☐	☐	☐

you have done a poo)

	None	Mild	Moderate	Severe
15. Difficulty swallowing fluids	☐	☐	☐	☐
16. Difficulty swallowing solids (feeling of foods sticking)	☐	☐	☐	☐
17. Pain on swallowing fluids	☐	☐	☐	☐
18. Pain on swallowing solids	☐	☐	☐	☐
19. Feeling fuller sooner than normal (feeling full up after a few mouthfuls)	☐	☐	☐	☐
20. Regurgitation of fluids (flow of fluids back into your mouth)	☐	☐	☐	☐
21. Regurgitation of solids (flow of food back into your mouth)	☐	☐	☐	☐
22. Faecal incontinence (accidents with bowels/ soiling/ some leakage of poo or fluid)	☐	☐	☐	☐

STEP 3 (to be completed by the ***study participant***)

In this step, we will talk about your **stools**. Please take a few moments to think about any problems you have had with your stools over the past 4 weeks (e.g. constipation, diarrhoea). Please indicate whether you have had problems during this time and how severe they were using the scale below.

	In the past month, I would rate the overall problems with my stools as: (please tick one)
1. **None:** I didn't have any problems	
2. **Mild:** I had some problems but they didn't bother me	
3. **Moderate:** I had problems often, they bothered me quite a bit	
4. **Severe:** I had a lot of problems, they bothered me a great deal	

The Bristol Stool Chart below helps to describe what your **stool consistency** has been like over the past 4 weeks. Refer to the chart when answering the questions.

Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. **Entirely Liquid**

A. In general, what stool type best describes your stools over the past 4 weeks, when you have been **"at your best"**? Please tick one.

Type ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

B. In general, what stool type best describes your stools over the past 4 weeks, when you have been **"at your worst"**? Please tick one.

Type ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

C. How often would you say you were **"at your best"** over the past 4 weeks? Please tick one.

☐ none of the time ☐ 1/4 of the time ☐ 1/2 of the time
☐ 3/4 of the time ☐ all of the time

D. How often would you say you are **"at your worst"** over the past 4 weeks? Please tick one.

☐ none of the time ☐ 1/4 of the time ☐ 1/2 of the time
☐ 3/4 of the time ☐ all of the time

8.5 CCR 3736 Modified Gastrointestinal Symptom Rating Scale

Patient Information: to be completed by the ***dietitian***   **Step 1, Step 2 and Step 3**: to be completed by the ***study participant***

Patient Information (*to be completed by the dietitian*)   Date: _____   Study number: _____   Is it a baseline measurement? ☐ yes ☐ no   Is it a follow-up measurement? ☐ yes ☐ no

STEP 1 (to be completed by the ***study participant***)

Please take a few moments to think about any **bowel symptoms** you have had over the past 2 weeks. Please indicate whether you have had bowel symptoms during this time and how severe they were using the scale below.

	In the past 2 weeks, I would rate my overall bowel symptoms as: (please tick one)
5. **None:** I didn't have any symptoms	
6. **Mild:** I had a/some symptoms but they didn't bother me	
7. **Moderate:** I had symptoms often, they bothered me quite a bit	
8. **Severe:** I had a lot of symptoms, they bothered me a great deal	

STEP 2 (to be completed by the ***study participant***)

Please rate your bowel symptoms over the past 2 weeks by placing a tick in the box that best describes your symptoms. (*Please tick 'none' if you did not have this symptom.)*

	I didn't have this symptom	**I had it a bit but it didn't bother me much**	**I had it often, it bothered me quite a bit**	**I had it a lot, it bothered me a great deal**
	None	**Mild**	**Moderate**	**Severe**
15. Abdominal pain / discomfort (any kind of pain in your belly)	☐	☐	☐	☐
16. Heartburn (burning / discomfort behind breastbone)	☐	☐	☐	☐
17. Acid reflux / acid regurgitation (flow of sour fluid into your mouth)	☐	☐	☐	☐
18. Nausea (feeling of wanting to be sick)	☐	☐	☐	☐
19. Abdominal rumbling / gurgling (bubbling or noise in the stomach)	☐	☐	☐	☐
20. Abdominal bloating / distension (swelling in the stomach or belly)	☐	☐	☐	☐
21. Belching / burping (bringing up gas through the mouth)	☐	☐	☐	☐
22. Flatulence / passing wind (release of gas from the bottom)	☐	☐	☐	☐
23. Constipation (stools are infrequent / difficult to pass)	☐	☐	☐	☐
24. Diarrhoea (loose or watery stools)	☐	☐	☐	☐
25. Loose stools (mushy or watery stools)	☐	☐	☐	☐
26. Hard stools (lumpy or dry stools)	☐	☐	☐	☐
27. Urgency to open bowels (rushing to do a poo)	☐	☐	☐	☐
28. Incomplete evacuation (feeling of something left behind after you have done a poo)	☐	☐	☐	☐

	None	Mild	Moderate	Severe
15. Difficulty swallowing fluids	☐	☐	☐	☐
16. Difficulty swallowing solids (feeling of foods sticking)	☐	☐	☐	☐
17. Pain on swallowing fluids	☐	☐	☐	☐
18. Pain on swallowing solids	☐	☐	☐	☐
19. Feeling fuller sooner than normal (feeling full up after a few mouthfuls)	☐	☐	☐	☐
20. Regurgitation of fluids (flow of fluids back into your mouth)	☐	☐	☐	☐
21. Regurgitation of solids (flow of food back into your mouth)	☐	☐	☐	☐
22. Faecal incontinence (accidents with bowels/ soiling/ some leakage of poo or fluid)	☐	☐	☐	☐
23. Wakening at night to do a poo	☐	☐	☐	☐
24. Fatty stools (floating, oily, sticky or smelly)	☐	☐	☐	☐
25. Change in frequency of stools (more stools than normal or less than normal)	☐	☐	☐	☐
26. Vomiting of solids or liquids	☐	☐	☐	☐

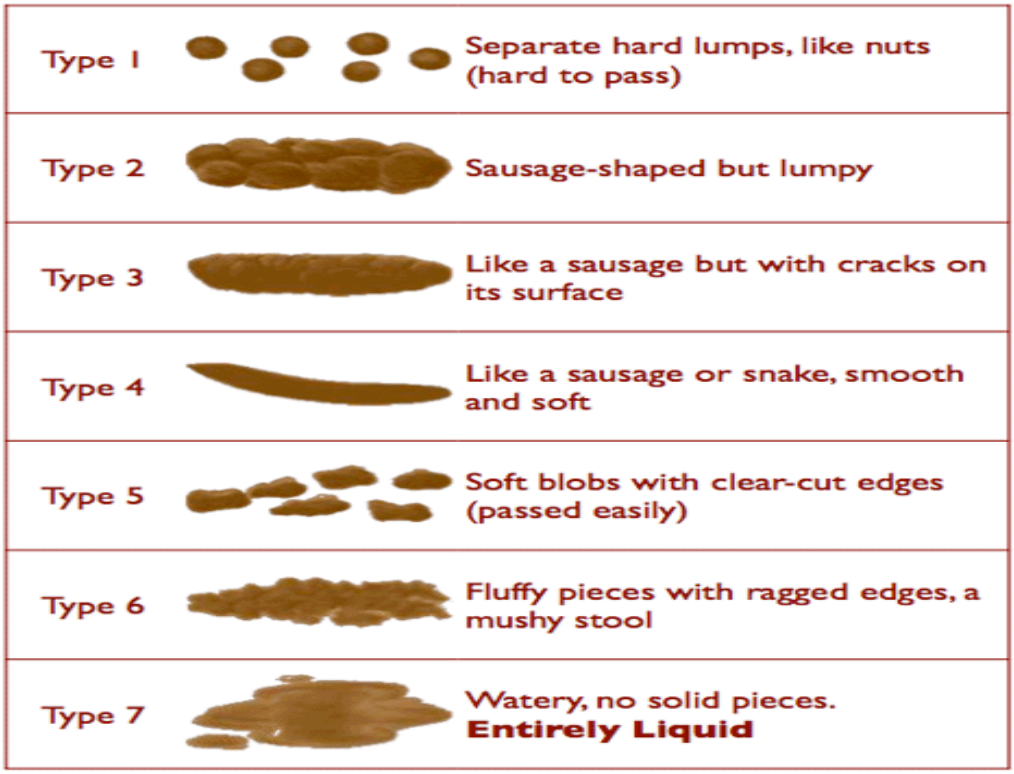
STEP 3 (to be completed by the ***study participant***)

In this step, we will talk about your **stools**. Please take a few moments to think about any problems you have had with your stools over the past 2 weeks (e.g. constipation, diarrhoea). Please indicate whether you have had problems during this time and how severe they were using the scale below.

	In the past 2 weeks, I would rate the overall problems with my stools as: (please tick one)
5. **None:** I didn't have any problems	
6. **Mild:** I had some problems but they didn't bother me	
7. **Moderate:** I had problems often, they bothered me quite a bit	
8. **Severe:** I had a lot of problems, they bothered me a great deal	

The Bristol Stool Chart below helps to describe what your **stool consistency** has been like over the past 2 weeks. Refer to the chart when answering the questions below.

Bristol Stool Chart



A. In general, what stool type best describes your stools over the past 2 weeks, when you have been **"at your best"**? Please tick one.

Type ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

B. In general, what stool type best describes your stools over the past 2 weeks, when you have been **"at your worst"**? Please tick one.

Type ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

B. How often would you say you were **"at your best"** over the past 2 weeks, Please tick one.

☐ none of the time ☐ 1/4 of the time ☐ 1/2 of the time
☐ 3/4 of the time ☐ all of the time

D. How often would you say you are **"at your worst"** over the past 2 weeks? Please tick one.

☐ none of the time ☐ 1/4 of the time ☐ 1/2 of the time
☐ 3/4 of the time ☐ all of the time

8.6 Malnutrition Universal Screening Tool

Reference: Elia 2003

BAPEN
Advancing Clinical Nutrition

'Malnutrition Universal Screening Tool'

MAG
Malnutrition Advisory Group
A Standing Committee of BAPEN

BAPEN is registered charity number 1023927 www.bapen.org.uk

'MUST'

'MUST' is a five-step screening tool to identify **adults,** who are malnourished, at risk of malnutrition (undernutrition), or obese. It also includes management guidelines which can be used to develop a care plan.

It is for use in hospitals, community and other care settings and can be used by all care workers.

This guide contains:

- A flow chart showing the 5 steps to use for screening and management
- BMI chart
- Weight loss tables
- Alternative measurements when BMI cannot be obtained by measuring weight and height.

The 5 'MUST' Steps

Step 1

Measure height and weight to get a BMI score using chart provided. *If unable to obtain height and weight, use the alternative procedures shown in this guide.*

Step 2

Note percentage unplanned weight loss and score using tables provided.

Step 3

Establish acute disease effect and score.

Step 4

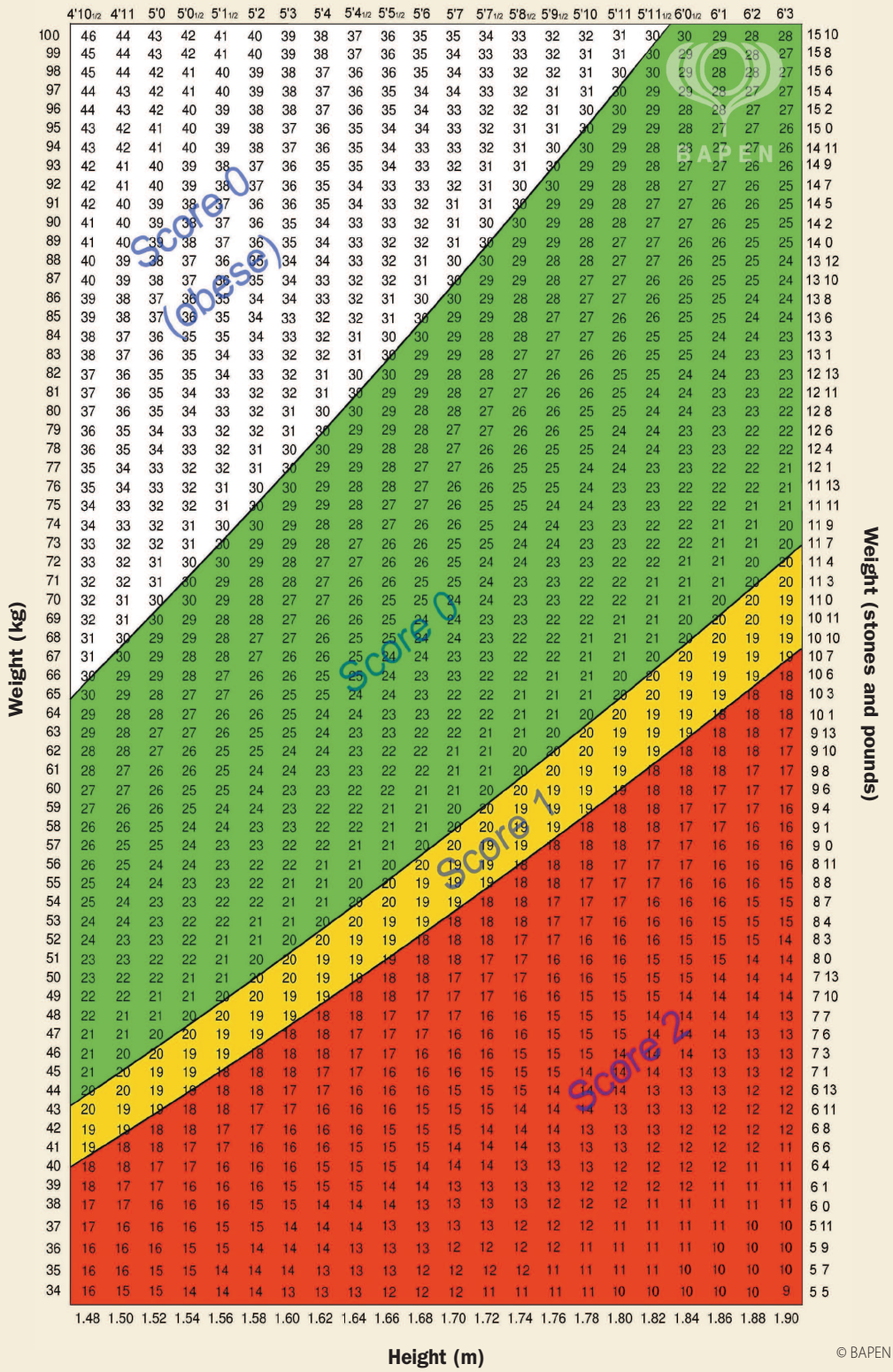
Add scores from steps 1, 2 and 3 together to obtain overall risk of malnutrition.

Step 5

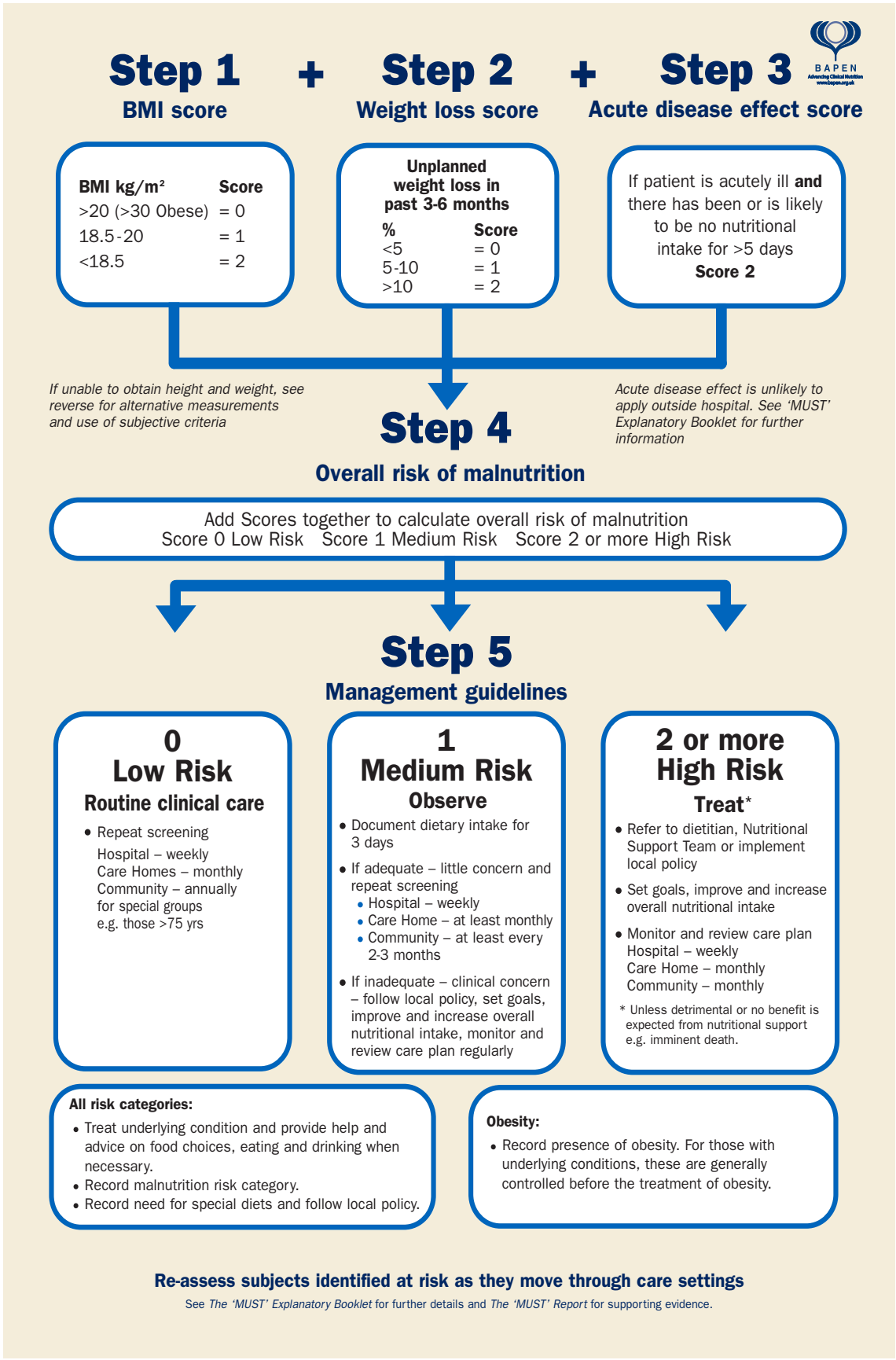
Use management guidelines and/or local policy to develop care plan.

Please refer to *The 'MUST' Explanatory Booklet* for more information when weight and height cannot be measured, and when screening patient groups in which extra care in interpretation is needed (e.g. those with fluid disturbances, plaster casts, amputations, critical illness and pregnant or lactating women). The booklet can also be used for training. See *The 'MUST' Report* for supporting evidence. Please note that 'MUST' has not been designed to detect deficiencies or excessive intakes of vitamins and minerals and is of **use only in adults.**

Step 1 – BMI score (& BMI)



Note : The black lines denote the exact cut off points (30,20 and 18.5 kg/m²), figures on the chart have been rounded to the nearest whole number.


Step 1
BMI score
+
Step 2
Weight loss score
+
Step 3
Acute disease effect score
BAPEN
BMI kg/m²
Score
>20 (>30 Obese) = 0
18.5-20 = 1
<18.5 = 2
Unplanned weight loss in past 3-6 months
%
Score
<5 = 0
5-10 = 1
>10 = 2
If patient is acutely ill and there has been or is likely to be no nutritional intake for >5 days
Score 2
If unable to obtain height and weight, see reverse for alternative measurements and use of subjective criteria
Acute disease effect is unlikely to apply outside hospital. See 'MUST' Explanatory Booklet for further information
Step 4
Overall risk of malnutrition
Add Scores together to calculate overall risk of malnutrition
Score 0 Low Risk Score 1 Medium Risk Score 2 or more High Risk
Step 5
Management guidelines
0
Low Risk
Routine clinical care
• Repeat screening
Hospital – weekly
Care Homes – monthly
Community – annually
for special groups
e.g. those >75 yrs
1
Medium Risk
Observe
• Document dietary intake for 3 days
• If adequate – little concern and repeat screening
• Hospital – weekly
• Care Home – at least monthly
• Community – at least every 2-3 months
• If inadequate – clinical concern – follow local policy, set goals, improve and increase overall nutritional intake, monitor and review care plan regularly
2 or more
High Risk
Treat*
• Refer to dietitian, Nutritional Support Team or implement local policy
• Set goals, improve and increase overall nutritional intake
• Monitor and review care plan
Hospital – weekly
Care Home – monthly
Community – monthly
* Unless detrimental or no benefit is expected from nutritional support e.g. imminent death.
All risk categories:
• Treat underlying condition and provide help and advice on food choices, eating and drinking when necessary.
• Record malnutrition risk category.
• Record need for special diets and follow local policy.
Obesity:
• Record presence of obesity. For those with underlying conditions, these are generally controlled before the treatment of obesity.
Re-assess subjects identified at risk as they move through care settings
See The 'MUST' Explanatory Booklet for further details and The 'MUST' Report for supporting evidence.

Step 2 – Weight loss score

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Weight before weight loss (kg)

	SCORE 0 Wt Loss < 5%	SCORE 1 Wt Loss 5-10%	SCORE 2 Wt Loss > 10%
34 kg	<1.70	1.70 – 3.40	>3.40
36 kg	<1.80	1.80 – 3.60	>3.60
38 kg	<1.90	1.90 – 3.80	>3.80
40 kg	<2.00	2.00 – 4.00	>4.00
42 kg	<2.10	2.10 – 4.20	>4.20
44 kg	<2.20	2.20 – 4.40	>4.40
46 kg	<2.30	2.30 – 4.60	>4.60
48 kg	<2.40	2.40 – 4.80	>4.80
50 kg	<2.50	2.50 – 5.00	>5.00
52 kg	<2.60	2.60 – 5.20	>5.20
54 kg	<2.70	2.70 – 5.40	>5.40
56 kg	<2.80	2.80 – 5.60	>5.60
58 kg	<2.90	2.90 – 5.80	>5.80
60 kg	<3.00	3.00 – 6.00	>6.00
62 kg	<3.10	3.10 – 6.20	>6.20
64 kg	<3.20	3.20 – 6.40	>6.40
66 kg	<3.30	3.30 – 6.60	>6.60
68 kg	<3.40	3.40 – 6.80	>6.80
70 kg	<3.50	3.50 – 7.00	>7.00
72 kg	<3.60	3.60 – 7.20	>7.20
74 kg	<3.70	3.70 – 7.40	>7.40
76 kg	<3.80	3.80 – 7.60	>7.60
78 kg	<3.90	3.90 – 7.80	>7.80
80 kg	<4.00	4.00 – 8.00	>8.00
82 kg	<4.10	4.10 – 8.20	>8.20
84 kg	<4.20	4.20 – 8.40	>8.40
86 kg	<4.30	4.30 – 8.60	>8.60
88 kg	<4.40	4.40 – 8.80	>8.80
90 kg	<4.50	4.50 – 9.00	>9.00
92 kg	<4.60	4.60 – 9.20	>9.20
94 kg	<4.70	4.70 – 9.40	>9.40
96 kg	<4.80	4.80 – 9.60	>9.60
98 kg	<4.90	4.90 – 9.80	>9.80
100 kg	<5.00	5.00 – 10.00	>10.00
102 kg	<5.10	5.10 – 10.20	>10.20
104 kg	<5.20	5.20 – 10.40	>10.40
106 kg	<5.30	5.30 – 10.60	>10.60
108 kg	<5.40	5.40 – 10.80	>10.80
110 kg	<5.50	5.50 – 11.00	>11.00
112 kg	<5.60	5.60 – 11.20	>11.20
114 kg	<5.70	5.70 – 11.40	>11.40
116 kg	<5.80	5.80 – 11.60	>11.60
118 kg	<5.90	5.90 – 11.80	>11.80
120 kg	<6.00	6.00 – 12.00	>12.00
122 kg	<6.10	6.10 – 12.20	>12.20
124 kg	<6.20	6.20 – 12.40	>12.40
126 kg	<6.30	6.30 – 12.60	>12.60

Weight before weight loss (st lb)

	SCORE 0 Wt Loss <5%	SCORE 1 Wt Loss 5-10%	SCORE 2 Wt Loss >10%
5st 4lb	<4lb	4lb – 7lb	>7lb
5st 7lb	<4lb	4lb – 8lb	>8lb
5st 11lb	<4lb	4lb – 8lb	>8lb
6st	<4lb	4lb – 8lb	>8lb
6st 4lb	<4lb	4lb – 9lb	>9lb
6st 7lb	<5lb	5lb – 9lb	>9lb
6st 11lb	<5lb	5lb – 10lb	>10lb
7st	<5lb	5lb – 10lb	>10lb
7st 4lb	<5lb	5lb – 10lb	>10lb
7st 7lb	<5lb	5lb – 11lb	>11lb
7st 11lb	<5lb	5lb – 11lb	>11lb
8st	<6lb	6lb – 11lb	>11lb
8st 4lb	<6lb	6lb – 12lb	>12lb
8st 7lb	<6lb	6lb – 12lb	>12lb
8st 11lb	<6lb	6lb – 12lb	>12lb
9st	<6lb	6lb – 13lb	>13lb
9st 4lb	<7lb	7lb – 13lb	>13lb
9st 7lb	<7lb	7lb – 13lb	>13lb
9st 11lb	<7lb	7lb – 1st 0lb	>1st 0lb
10st	<7lb	7lb – 1st 0lb	>1st 0lb
10st 4lb	<7lb	7lb – 1st 0lb	>1st 0lb
10st 7lb	<7lb	7lb – 1st 1lb	>1st 1lb
10st 11lb	<8lb	8lb – 1st 1lb	>1st 1lb
11st	<8lb	8lb – 1st 1lb	>1st 1lb
11st 4lb	<8lb	8lb – 1st 2lb	>1st 2lb
11st 7lb	<8lb	8lb – 1st 2lb	>1st 2lb
11st 11lb	<8lb	8lb – 1st 3lb	>1st 3lb
12st	<8lb	8lb – 1st 3lb	>1st 3lb
12st 4lb	<9lb	9lb – 1st 3lb	>1st 3lb
12st 7lb	<9lb	9lb – 1st 4lb	>1st 4lb
12st 11lb	<9lb	9lb – 1st 4lb	>1st 4lb
13st	<9lb	9lb – 1st 4lb	>1st 4lb
13st 4lb	<9lb	9lb – 1st 5lb	>1st 5lb
13st 7lb	<9lb	9lb – 1st 5lb	>1st 5lb
13st 11lb	<10lb	10lb – 1st 5lb	>1st 5lb
14st	<10lb	10lb – 1st 6lb	>1st 6lb
14st 4lb	<10lb	10lb – 1st 6lb	>1st 6lb
14st 7lb	<10lb	10lb – 1st 6lb	>1st 6lb
14st 11lb	<10lb	10lb – 1st 7lb	>1st 7lb
15st	<11lb	11lb – 1st 7lb	>1st 7lb
15st 4lb	<11lb	11lb – 1st 7lb	>1st 7lb
15st 7lb	<11lb	11lb – 1st 8lb	>1st 8lb
15st 11lb	<11lb	11lb – 1st 8lb	>1st 8lb
16st	<11lb	11lb – 1st 8lb	>1st 8lb
16st 4lb	<11lb	11lb – 1st 9lb	>1st 9lb
16st 7lb	<12lb	12lb – 1st 9lb	>1st 9lb

Alternative measurements and considerations

Step 1: BMI (body mass index)

If height cannot be measured

- Use recently documented or self-reported height (if reliable and realistic).
- If the subject does not know or is unable to report their height, use one of the alternative measurements to estimate height (ulna, knee height or demispan).

Step 2: Recent unplanned weight loss

If recent weight loss cannot be calculated, use self-reported weight loss (if reliable and realistic).

Subjective criteria

If height, weight or BMI cannot be obtained, the following criteria which relate to them can assist your professional judgement of the subject's nutritional risk category. Please note, these criteria should be used collectively not separately as alternatives to steps 1 and 2 of 'MUST' and are not designed to assign a score. Mid upper arm circumference (MUAC) may be used to estimate BMI category in order to support your overall impression of the subject's nutritional risk.

1. BMI

- Clinical impression – thin, acceptable weight, overweight. Obvious wasting (very thin) and obesity (very overweight) can also be noted.

2. Unplanned weight loss

- Clothes and/or jewellery have become loose fitting (weight loss).
- History of decreased food intake, reduced appetite or swallowing problems over 3-6 months and underlying disease or psycho-social/physical disabilities likely to cause weight loss.

3. Acute disease effect

- Acutely ill and no nutritional intake or likelihood of no intake for more than 5 days.

Further details on taking alternative measurements, special circumstances and subjective criteria can be found in *The 'MUST' Explanatory Booklet*. A copy can be downloaded at www.bapen.org.uk or purchased from the BAPEN office. The full evidence-base for 'MUST' is contained in *The 'MUST' Report* and is also available for purchase from the BAPEN office.

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bapen@sovereignconference.co.uk BAPEN is registered charity number 1023927. www.bapen.org.uk

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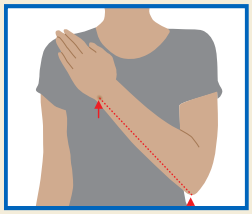
'MUST' is supported by the British Dietetic Association, the Royal College of Nursing and the Registered Nursing Home Association.

Alternative measurements: instructions and tables

If height cannot be obtained, use length of forearm (ulna) to calculate height using tables below. *(See The 'MUST' Explanatory Booklet for details of other alternative measurements (knee height and demispan) that can also be used to estimate height).*

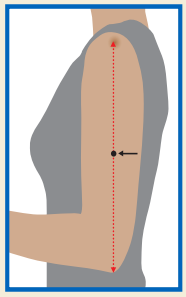
Estimating height from ulna length



Measure between the point of the elbow (olecranon process) and the midpoint of the prominent bone of the wrist (styloid process) (left side if possible).

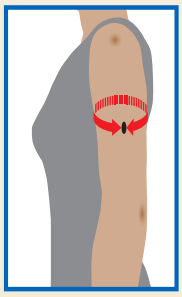
HEIGHT (m)	Men (<65 years)	1.94	1.93	1.91	1.89	1.87	1.85	1.84	1.82	1.80	1.78	1.76	1.75	1.73	1.71
	Men (≥65 years)	1.87	1.86	1.84	1.82	1.81	1.79	1.78	1.76	1.75	1.73	1.71	1.70	1.68	1.67
	Ulna length (cm)	32.0	31.5	31.0	30.5	30.0	29.5	29.0	28.5	28.0	27.5	27.0	26.5	26.0	25.5
HEIGHT (m)	Women (<65 years)	1.84	1.83	1.81	1.80	1.79	1.77	1.76	1.75	1.73	1.72	1.70	1.69	1.68	1.66
	Women (≥65 years)	1.84	1.83	1.81	1.79	1.78	1.76	1.75	1.73	1.71	1.70	1.68	1.66	1.65	1.63
HEIGHT (m)	Men (<65 years)	1.69	1.67	1.66	1.64	1.62	1.60	1.58	1.57	1.55	1.53	1.51	1.49	1.48	1.46
	Men (≥65 years)	1.65	1.63	1.62	1.60	1.59	1.57	1.56	1.54	1.52	1.51	1.49	1.48	1.46	1.45
	Ulna length (cm)	25.0	24.5	24.0	23.5	23.0	22.5	22.0	21.5	21.0	20.5	20.0	19.5	19.0	18.5
HEIGHT (m)	Women (<65 years)	1.65	1.63	1.62	1.61	1.59	1.58	1.56	1.55	1.54	1.52	1.51	1.50	1.48	1.47
	Women (≥65 years)	1.61	1.60	1.58	1.56	1.55	1.53	1.52	1.50	1.48	1.47	1.45	1.44	1.42	1.40

Estimating BMI category from mid upper arm circumference (MUAC)



The subject's left arm should be bent at the elbow at a 90 degree angle, with the upper arm held parallel to the side of the body. Measure the distance between the bony protrusion on the shoulder (acromion) and the point of the elbow (olecranon process). Mark the mid-point.

Ask the subject to let arm hang loose and measure around the upper arm at the mid-point, making sure that the tape measure is snug but not tight.

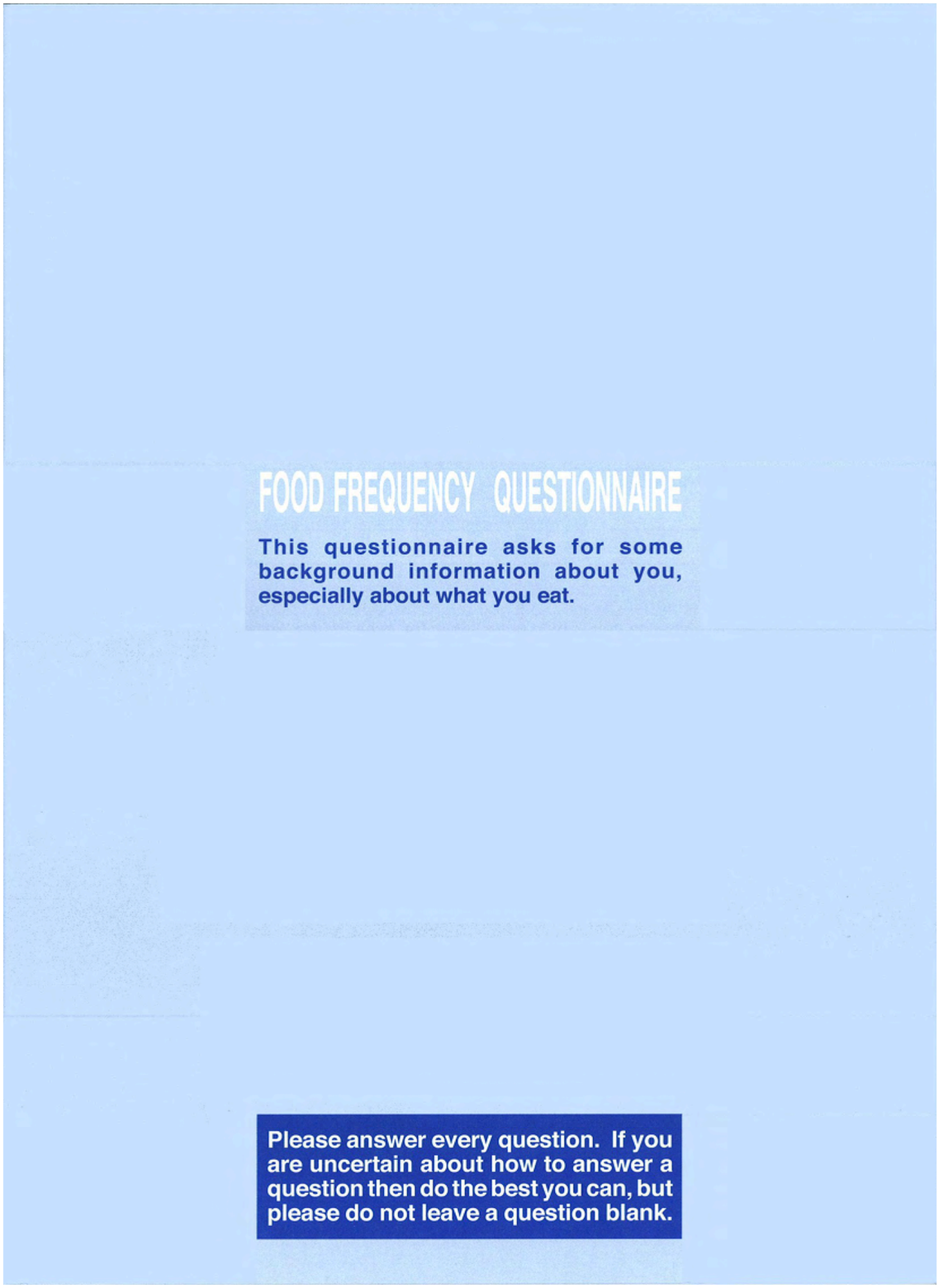


If MUAC is <23.5 cm, BMI is likely to be <20 kg/m^2.
If MUAC is >32.0 cm, BMI is likely to be >30 kg/m^2.

The use of MUAC provides a general indication of BMI and is not designed to generate an actual score for use with 'MUST'. For further information on use of MUAC please refer to *The 'MUST' Explanatory Booklet.*

8.7 European Prospective Investigation into Cancer-Norfolk Food Frequency Questionnaire Version 6

Reference: Bingham 1997



1. **YOUR DIET LAST YEAR**

For each food there is an amount shown, either a "medium serving" or a common household unit such as a slice or teaspoon. Please put a tick (✓) in the box to indicate how often, **on average**, you have eaten the specified amount of each food **during the past year**.

EXAMPLES:

For white bread the amount is one slice, so if you ate 4 or 5 slices a day, you should put a tick in the column headed "4-5 per day".

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
BREAD AND SAVOURY BISCUITS (one slice or biscuit)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread and rolls								✓	

For chips, the amount is a "medium serving", so if you had a helping of chips twice a week you should put a tick in the column headed "2-4 per week".

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
POTATOES, RICE AND PASTA (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Chips				✓					

For very seasonal fruits such as strawberries and raspberries you should estimate your average use when the fruits are in season, so if you ate strawberries or raspberries about once a week when they were in season you should put a tick in the column headed "once a week"

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
FRUIT (1 fruit or medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Strawberries, raspberries, kiwi fruit			✓						

Please estimate your average food use as best you can, and please answer every question – do not leave ANY lines blank. PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
MEAT AND FISH (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Beef: roast, steak, mince, stew or casserole									
Beefburgers									
Pork: roast, chops, stew or slices									
Lamb: roast, chops or stew									
Chicken or other poultry eg. turkey									
Bacon									
Ham									
Corned beef, Spam, luncheon meats									
Sausages									
Savoury pies, eg. meat pie, pork pie, pasties, steak & kidney pie, sausage rolls									
Liver, liver paté, liver sausage									
Fried fish in batter, as in fish and chips									
Fish fingers, fish cakes									
Other white fish, fresh or frozen, eg. cod, haddock, plaice, sole, halibut									
Oily fish, fresh or canned, eg. mackerel, kippers, tuna, salmon, sardines, herring									
Shellfish, eg. crab, prawns, mussels									
Fish roe, taramasalata									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

This food frequency questionnaire (CAMB/PQ/6/1205) was originally designed for the EPIC-Norfolk Study.
www.epic-norfolk.org.uk

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
BREAD AND SAVOURY BISCUITS (one slice or biscuit)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread and rolls									
Brown bread and rolls									
Wholemeal bread and rolls									
Cream crackers, cheese biscuits									
Crispbread, eg. Ryvita									
CEREALS (one bowl)									
Porridge, Readybrek									
Breakfast cereal such as cornflakes, muesli etc.									
POTATOES, RICE AND PASTA (medium serving)									
Boiled, mashed, instant or jacket potatoes									
Chips									
Roast potatoes									
Potato salad									
White rice									
Brown rice									
White or green pasta, eg. spaghetti, macaroni, noodles									
Wholemeal pasta									
Lasagne, moussaka									
Pizza									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

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FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
DAIRY PRODUCTS AND FATS	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Single or sour cream (tablespoon)									
Double or clotted cream (tablespoon)									
Low fat yogurt, fromage frais (125g carton)									
Full fat or Greek yogurt (125g carton)									
Dairy desserts (125g carton)									
Cheese, eg. Cheddar, Brie, Edam (medium serving)									
Cottage cheese, low fat soft cheese (medium serving)									
Eggs as boiled, fried, scrambled, etc. (one)									
Quiche (medium serving)									
Low calorie, low fat salad cream(tablespoon)									
Salad cream, mayonnaise (tablespoon)									
French dressing (tablespoon)									
Other salad dressing (tablespoon)									
The following on bread or vegetables									
Butter (teaspoon)									
Block or hard margarine, eg. Stork, Krona (teaspoon)									
Polyunsaturated margarine, eg. Flora, sunflower, soya spreads (teaspoon)									
Soft margarines,including olive oil based and dairy spreads, eg. Blue Band, Olivio/ Bertolli, Clover (teaspoon)									
Low fat spreads (less than 60% fat), eg. Outline, Gold (teaspoon)									
Very low fat spread (less than 30% fat) (teaspoon)									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

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PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
SWEETS AND SNACKS (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Sweet biscuits, chocolate , eg. digestive (one)									
Sweet biscuits, plain, eg. Nice, ginger (one)									
Cakes eg. fruit, sponge, home baked									
Cakes eg. fruit, sponge, ready made									
Buns, pastries eg. scones, flapjacks, home baked									
Buns, pastries eg. croissants, doughnuts, ready made									
Fruit pies, tarts, crumbles, home baked									
Fruit pies, tarts, crumbles, ready made									
Sponge puddings, home baked									
Sponge puddings, ready made									
Milk puddings, eg. rice, custard, trifle									
Ice cream, choc ices									
Chocolates, single or squares									
Chocolate snack bars eg. Mars, Crunchie									
Sweets, toffees, mints									
Sugar added to tea, coffee, cereal (teaspoon)									
Crisps or other packet snacks, eg. Wotsits									
Peanuts or other nuts									
SOUPS, SAUCES, AND SPREADS									
Vegetable soups (bowl)									
Meat soups (bowl)									
Sauces, eg. white sauce, cheese sauce, gravy (tablespoon)									
Tomato ketchup (tablespoon)									
Pickles, chutney (tablespoon)									
Marmite, Bovril (teaspoon)									
Jam, marmalade, honey (teaspoon)									
Peanut butter (teaspoon)									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

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PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
DRINKS	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Tea (cup)									
Coffee, instant or ground (cup)									
Coffee, decaffeinated (cup)									
Coffee whitener, eg. Coffee-mate (teaspoon)									
Cocoa, hot chocolate (cup)									
Horlicks, Ovaltine (cup)									
Wine (glass)									
Beer, lager or cider (half pint)									
Port, sherry, vermouth, liqueurs (glass)									
Spirits, eg. gin, brandy, whisky, vodka (single)									
Low calorie or diet fizzy soft drinks (glass)									
Fizzy soft drinks, eg. Coca cola, lemonade (glass)									
Pure fruit juice (100%) eg. orange, apple juice (glass)									
Fruit squash or cordial (glass)									
FRUIT **For seasonal fruits marked *, please estimate your average use when the fruit is in season**									
Apples (1 fruit)									
Pears (1 fruit)									
Oranges, satsumas, mandarins (1 fruit)									
Grapefruit (half)									
Bananas (1 fruit)									
Grapes (medium serving)									
Melon (1 slice)									
* Peaches, plums, apricots (1 fruit)									
* Strawberries, raspberries, kiwi fruit (medium serving)									
Tinned fruit (medium serving)									
Dried fruit, eg. raisins, prunes (medium serving)									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

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PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
VEGETABLES Fresh, frozen or tinned (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Carrots									
Spinach									
Broccoli, spring greens, kale									
Brussels sprouts									
Cabbage									
Peas									
Green beans, broad beans, runner beans									
Marrow, courgettes									
Cauliflower									
Parsnips, turnips, swedes									
Leeks									
Onions									
Garlic									
Mushrooms									
Sweet peppers									
Beansprouts									
Green salad, lettuce, cucumber, celery									
Watercress									
Tomatoes									
Sweetcorn									
Beetroot									
Coleslaw									
Avocado									
Baked beans									
Dried lentils, beans, peas									
Tofu , soya meat, TVP, Vegeburger									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

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YOUR DIET LAST YEAR, continued

2. Are there any **OTHER** foods which you ate more than once a week? Yes ☐ No ☐

If **YES,** please list below

Food	Usual serving size	Number of times eaten each week

3. What type of milk did you most often use?

Select one only

Full cream/whole ☐ Semi-skimmed ☐
Skimmed ☐ Channel Islands, gold ☐
Dried milk ☐ Soya ☐
Other, specify ☐ None ☐

4. How much milk did you drink each day, including milk with tea, coffee, cereals etc?

None ☐ Three quarters of a pint ☐
Quarter of a pint ☐ One pint ☐
Half a pint ☐ More than one pint ☐

5. Did you usually eat breakfast cereal (excluding porridge and Ready Brek mentioned earlier)? Yes ☐ No ☐

If **YES,** which brand and type of breakfast cereal, including muesli, did you usually eat?

List the one or two types most often used

Brand e.g. Kellogg's	Type e.g. cornflakes

6. What kind of fat did you most often use for frying, roasting, grilling etc?

Select one only

Butter ☐ Solid vegetable fat ☐
Lard/dripping ☐ Margarine ☐
Vegetable oil ☐ None ☐

If you used vegetable oil, please give type eg. corn, sunflower ☐

7. What kind of fat did you most often use for baking cakes etc?

Select one only

Butter ☐ Solid vegetable fat ☐
Lard/dripping ☐ Margarine ☐
Vegetable oil ☐ None ☐

If you used margarine, please give name or type eg. Flora, Stork ☐

8. How often did you eat food that was fried at home?

Daily ☐ 1-3 times a week ☐ 4-6 times a week ☐
Less than once a week ☐ Never ☐

9. How often did you eat fried food away from home?

Daily ☐ 1-3 times a week ☐ 4-6 times a week ☐
Less than once a week ☐ Never ☐

10. What did you do with the visible fat on your meat?

Ate most of the fat ☐ Ate as little as possible ☐
Ate some of the fat ☐ Did not eat meat ☐

11. How often did you eat grilled or roast meat? ☐☐ times a week

12. How well cooked did you usually have grilled or roast meat?

Well done /dark brown ☐ Lightly cooked/rare ☐
Medium ☐ Did not eat meat ☐

13. How often did you add salt to food while cooking?

Always ☐ Rarely ☐
Usually ☐ Never ☐
Sometimes ☐

14. How often did you add salt to any food at the table?

Always ☐ Rarely ☐
Usually ☐ Never ☐
Sometimes ☐

15. Did you regularly use a salt substitute (eg LoSalt)? Yes ☐ No ☐

If **YES**, which brand? _____

16. During the course of last year, on average, how many times a week did you eat the following foods?

Food type	**Times/week**	**Portion size**
Vegetables (not including potatoes)	☐☐	medium serving
Salads	☐☐	medium serving
Fruit and fruit products (not including fruit juice)	☐☐	medium serving or 1 fruit
Fish and fish products	☐☐	medium serving
Meat, meat products and meat dishes (including bacon, ham and chicken)	☐☐	medium serving

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17. Have you taken any vitamins, minerals, fish oils, fibre or other food supplements during the past year?

- ☐ Yes
- ☐ No
- ☐ Sometimes
- ☐ Don't know

If **YES or SOMETIMES,** please complete the table below.

If you have taken more than 8 types of supplement please put the most frequently consumed brands first.

Example: If you take one tablet of vitamin C two times a day, please write '2' in the amount-column and tick (✓) the 'once a day' box. Most supplements mention a strength value (in our example 500mg), please write this information in the table.

Supplements				Average frequency for the past year Tick (✓) **ONE** box per line to show how often on average you took the amount consumed as mentioned in 'amount' column.					
Brand	Name	Strength (strength of the supple-ment for each tablet or capsule)	Amount (number of tablets, capsules or teaspoons taken in one day)	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day
Boots	High strength vitamin C	500mg	2 tablets	EXAMPLE					✓

Thank you for your help

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8.8 Glucose Hydrogen Methane Breath Test Information Booklet

Reference: The Royal Marsden NHS Foundation Trust 2012

The ROYAL MARSDEN
NHS Foundation Trust

Glucose Hydrogen/Methane Breath Testing

Endoscopy Unit

Patient Information

NHS

What is a breath test?

A glucose hydrogen breath test is used to make several diagnoses including lactose intolerance, carbohydrate malabsorption and small bowel bacterial overgrowth.

After giving you a sugary-tasting drink, we will ask you to blow (exhale) into a small bag and measure the gases in your breath every 20 minutes until the test has been completed. Please allow 3 hours to be at the hospital.

Why am I having a breath test?

If the working of your digestive tract has been changed by surgery, chemotherapy, radiotherapy or other conditions, you may have symptoms such as watery loose stool, a need to rush to the lavatory, wind and bloating.

If certain gases (hydrogen and methane) in your breath are abnormally high, it will help us to establish whether your symptoms are due to specific foods in your diet or whether you have germs in the small bowel where there should not be any. In small bowel bacterial overgrowth, the test is used to try and detect the presence of germs in the small bowel.

What preparation will I need for my breath test?

You will be asked to change your diet (as outlined in this leaflet) so that any breath test measurements recorded are accurate. It is important that you follow the instructions below very carefully. If these are not followed, your procedure may have to be cancelled.

If any of the following apply to you, please contact the endoscopy suite a few days prior to your appointment (contact details on last page):

- You have any concerns/queries
- You have diabetes
- You are taking chemotherapy drugs
- You are taking anti-epileptic drugs
- You are taking a medication which is taken daily at a set time
- You are due to undergo a gastroscopy and/or colonoscopy on the same day as the breath test procedure

24 hours before the test

Foods from the following list are allowed to be eaten and does not influence the test result:

- Red meat
- Fish eg. white fish, shellfish, tuna, salmon,
- Chicken
- Tofu, Quorn
- Eggs – scrambled, boiled, fried, poached
- Cheese – all types
- Milk, natural yoghurt, ice cream
- White: bread / rolls / croissants / chapattis/ rotis / naan / pitta bread / pastry
- White pasta or rice
- Rice crispies, cornflakes, congee
- Rich tea biscuits/other plain biscuits
- Oil, butter, margarine, ghee
- Potato (no skin) eg. boiled, mashed, roast, crisps

Tea/Coffee with a splash of milk & no sugar. Herbal tea.

- Salt / pepper / herbs / spices / marmite / mayonnaise / mustard / salad dressing
- Sugar free chewing gum

The following food does influence the test result and should NOT be consumed for 24 hours before the test:

- Canned drinks, carbonated drinks & fruit juices
- Alcohol
- Fruit (including fresh, tinned, stewed, dried, or preserved)
- ALL vegetables except potatoes (no skin)
- Sweets, chocolate
- Sugar
- Marmalade, jam, honey, chocolate spread, peanut butter
- Tomato Ketchup, brown sauce, pickle, chutney, chilli sauce
- Wholegrain cereals eg. weetabix, all bran, bran flakes, muesli
- Brown rice or pasta

- Wholemeal: bread / rolls / chapattis/ rotis / naan
- Lentils, pulses
- Nuts

12 hours before the test:

- Please do not eat or drink anything except water for 12 hours before the test ie. if your test is at 8am, stop eating and drinking after 8pm on the previous night.
- You are allowed to drink water at any time.
- Take your evening medications as usual.

On the morning of your test

- Please clean your teeth. Avoid mouthwash unless it is sugar free. Sugar free mouthwash will be provided at the endoscopy suite.
- Unless told otherwise, DO NOT take your usual medication (as it may be sugar coated) before the test, however do bring ALL your usual medication with you to the hospital so you can take it after completion of the test.
- Do not smoke for an hour before the test or during the test as it raises your hydrogen levels and causes a false positive result.

What will happen when I come up for the breath test?

- You will be asked to complete a questionnaire about your symptoms.
- You will be asked to blow (exhale) into a bag for a baseline measurement.
- You will be given a small sweet liquid to drink.
- You will then be asked to blow (exhale) in to a bag at specific times until the test is completed.
- You will be asked to write down any bowel symptoms you experience during the test.

What happens afterwards?

You may eat and drink as normal. You will be given a drink and a sandwich following the procedure and can take your usual medications.

When will I know the results?

The results will be sent to your GP and the consultant who referred you for the test. A follow up appointment will be arranged if required.

Endoscopy Suite Contact Details

The working times of the Endoscopy Suite are 08.00 - 17.00 Monday to Friday: **0207 811 8328**. If your call is unanswered, you can leave an answerphone message. Answerphone messages will be checked twice daily (Monday – Friday) and a member of the Endoscopy Suite will return your call as soon as possible.

Outside of working hours, you can ring the main switchboard number: 0207 352 8171 and ask to speak to the Clinical Site Practitioner (bleep 022) at Chelsea.

If you would like this information sheet in a different format, please contact the PALs office on 0800 783 7176 or talk to the clinical staff responsible for your care.

References

This booklet is evidence based wherever the appropriate evidence is available, and represents an accumulation of expert opinion and professional interpretation.

Details of the references used in writing this booklet are available on request from:

The Royal Marsden Help Centre

Freephone: 0800 783 7176

Email: patientcentre@rmh.nhs.uk

No conflicts of interest were declared in the production of this booklet

Published October 2012. Planned review October 2014

Life demands excellence

8.9 CCR 3703 Ethical Approval Letter

NHS
National Research Ethics Service
NRES Committee London - Riverside
South West Research Ethics Committee
Level 3 Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT

Telephone: 01173421385
Facsimile: 01173420445

31 October 2011

Dr Jervoise Andreyev
Consultant Gastroenterologist in Pelvic Radiation Disease
The Royal Marsden NHS Foundation Trust
GI Unit, The Royal Marsden NHS Foundation Trust,
Fulham Rd
London
SW3 6JJ

Dear Dr Andreyev

Full title of study: **An observational study to assess the prevalence and profile of oesophago-gastric cancer patients who develop small intestinal bacterial overgrowth during or after cancer treatment**
REC reference number: **11/LO/1583**
Protocol number: **CCR3703**
EudraCT number:

Thank you for your letter of 17 October 2011. I can confirm the REC has received the documents listed below as evidence of compliance with the approval conditions detailed in our letter dated 03 October 2011. Please note these documents are for information only and have not been reviewed by the committee.

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Covering Letter		17 October 2011
Participant Information Sheet: Patient Information Sheet	1	20 July 2011

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

11/LO/1583 **Please quote this number on all correspondence**

Yours sincerely

Miss Stephanie Macpherson

This Research Ethics Committee is an advisory committee to the South Central Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

Committee Co-ordinator

E-mail: ubh-tr.RiversideREC@nhs.net

Copy to: *Ms Eva Grace*
eva.grace@rmh.nhs.uk

Ms Jane Lawrence, Royal Marsden NHS Foundation Trust
jane.lawrence@rmh.nhs.uk

8.10 CCR 3703 Patient Information Sheet

The ROYAL MARSDEN
NHS Foundation Trust

'Monitoring nutrition and gastrointestinal symptoms during and after treatment for disorders of the oesophagus and stomach'

Introduction

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Ask us if there is anything, which is unclear to you, or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for taking the time to read this.

Why have I been invited to take part?

You have been invited to take part in this study because you are a patient at The Royal Marsden NHS Foundation Trust. You have just been diagnosed with a disorder of the oesophagus (gullet) or stomach and after discussion with you, an appropriate treatment pathway has been chosen for you. This study will recruit men and women who will undergo surgery and/or receive radiotherapy/chemotherapy for disorders of the oesophagus or stomach. Therefore, you fit the criteria for the patient-type we are looking for.

What is the purpose of the study?

The study has objectives, which include gathering information on the symptoms and nutritional issues that patients may experience after their diagnosis. Also, in some patients, we will be collecting data on the number of patients who develop a condition called small bowel bacterial overgrowth during and after treatment for their disorder.

Symptoms and nutritional issues caused by treatment

As a result of your disorder and the treatment you will receive for it, you may develop symptoms and nutritional issues. The symptoms may include abdominal pain/discomfort, nausea, reflux, difficult swallowing, diarrhoea or constipation. One or more of these symptoms may develop in the early stages of your treatment and/or continue for months after you have finished it. We would like to find out more about the specific symptoms which patients experience, as they can affect many aspects of your life, including your nutritional status.

The nutritional issues, which patients may face when undergoing treatment for their condition may include difficulty swallowing, reflux, nausea, vomiting, and changes in taste and food preferences. In addition, patients may become malnourished. Malnutrition is a word used to describe a condition resulting from poor or inadequate food intake. Patients can become malnourished for many reasons e.g. as a result of

having the symptoms mentioned above, the effect of your cancer or reduced appetite. As part of this study, you will be assessed for malnutrition on a regular basis. This will provide us with information on the numbers of patients who become malnourished.

The numbers of patients who develop small bowel bacterial overgrowth
Firstly, a brief background on what small bowel bacterial overgrowth is. In most healthy people, very few bacteria are able to survive in the upper part of the small bowel. It is usually a very sterile area. Nearly all of the bacteria living along the gastrointestinal tract (gut) live in the large bowel. Their role here is very important for our health. Sometimes, often in illness, these bacteria move upwards into the small bowel, where they can multiply. If they reach large enough numbers, they start to cause symptoms e.g. diarrhoea, bloating/gasiness, weight loss and nutritional deficiencies. This is called 'small bowel bacterial overgrowth'.

There are many reasons why small bowel bacterial overgrowth can occur in patients like you. It may develop following surgery, where the anatomy of the gastrointestinal tract has been changed. Patients who receive chemotherapy and/or radiotherapy may also be at risk of developing it, as the healthy cells of the gut may become damaged and so bacterial numbers can increase. However, to date, there has been very little research looking at the condition in patients undergoing treatment for oesophageal and gastric disorders. By finding out how often the condition occurs, and in which patients, we hope that we can gather information to allow early detection and so improve the care of these patients.

Do I have to take part?
It is up to you whether or not you decide to join this study. We will describe the study and go through this information sheet with you. If you agree to take part we will then ask you to sign a consent form. You are free to withdraw at any time without giving a reason. Your usual treatment will not be affected in any way.

What will happen to me if I take part?
If you decide to take part you will be seen by a dietitian before you begin your treatment – she will see you throughout the duration of the study. Whenever possible, this will be on the same days that you have other appointments at the Royal Marsden Hospital. You will see her three times in total; at the start of your treatment, after 3-months and then after another 9-months. Each appointment will include a glucose breath test.

The glucose breath test, a simple, low risk procedure, is used to get an idea of the number of bacteria in your small bowel by measuring the gases in your breath. It helps with diagnosing small bowel bacterial overgrowth. The test will take place in the morning and you will be asked to have been fasting since the night before. The dietitian (or a nurse) will give you a sugary liquid and will ask you to drink it. You will then breathe into a bag once every 20 minutes for the next 1.5 hours (maximum of 2.5 hours) and your breath will be analysed using a machine.

At each appointment with the dietitian, you will be asked to fill in some questionnaires. One questionnaire will ask you about your symptoms and another about your food intake. In addition, the dietitian will ask you questions about your nutrition, which will identify malnutrition if it is present. She will also take your temperature and examine you to assess your muscle and fat stores. On average, each appointment should take approximately 1.5 hours in total.

What are the possible risks and disadvantages of taking part?
This study is low risk as the breath test is non-invasive and the questionnaires will just involve you answering the questions asked. The examination component of the study requires you to be able to stand or sit on scales to be weighed and involves you undergoing an examination while lying in bed or sitting in a chair. Before any examination is carried out the dietitian will speak to you and assess if you would experience any difficulty being examined in this way. All examinations would be done in private with you wearing your own clothes.

What are the possible benefits of taking part?
There are two potential benefits of this study for you. As we are specifically looking at the development of small bowel bacterial overgrowth, should you at any stage develop symptoms of it, we will ask your medical team to look into it. Therefore, we could pick up on a case of small bowel bacterial overgrowth or another condition much earlier (and so treat it earlier) than if you had not taken part in the study. Similarly, as we will be using questions and measurements to detect malnutrition, we can quickly recognize if you have or are at risk of becoming malnourished. You will then be referred to a dietitian for advice on your diet. We also hope the study may benefit patients in the future as it will allow us to gather invaluable data, which in the longer term will allow us to deliver improved care to them.

What happens when the research study stops?
Your normal care will continue in the usual way.

Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be kept confidential.

Who is organising and funding the research?
The Royal Marsden NHS Foundation Trust is sponsoring this research.

Who has reviewed the study?
The Royal Marsden Committee for Clinical Research (CCR) and the London-Riverside National Research Ethics Committee (NREC).

Further Information

Before you make a decision about your participation in this study, remember that you can ask us any questions. Allow yourself as much time as you need to think through your decision. If you then decide that you still wish to take part, you will be asked to confirm in writing that you have read and understand this patient information and that all of your questions have been answered completely and that you wish to continue in the study. If you would like further information about the study, or have any concerns during the study, please contact Eva Grace, Research Dietitian, Tel. 020 7352 8171 (ext.4653) or Dr. Jervoise Andreyev, Consultant Gastroenterologist in Pelvic Radiation Disease, Tel. 020 7811 8216, who will be happy to discuss the study with you.

8.11 CCR 3703 Consent Form

The ROYAL MARSDEN
NHS Foundation Trust

Study Protocol Number:
Ethics Protocol Number:
Patient Identification Number for this trial:

Study title: "An observational study to assess the prevalence and profile of oesophagogastric cancer patients who develop small intestinal bacterial overgrowth during or after cancer treatment"

Name of Principal Investigator: Dr. Jervoise Andreyev

1. I confirm that I have read and understand the information sheet dated 10th Oct 2012 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and my medical care and legal rights will not be affected. ☐
3. I give permission for individuals from regulatory authorities or from The Royal Marsden NHS Foundation Trust, to have access to relevant sections of my medical notes and data collected during the study ☐
4. I consent to the researcher keeping the anonymous data in a locked office for a maximum of fifteen years post commencement of the study for confirmation purposes during preparation for publication. ☐
5. I have been assured strict confidentiality will be maintained. ☐
6. I agree to my GP being informed of my participation in the study. ☐
7. I agree to take part in the above study. ☐
8. I would/ would not like to be informed of the results of this study. Please circle one.

Please sign here:

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Signature of Participant	Date	Print Name
----------	----------	----------
Signature of Researcher	Date	Print Name

8.12 Subjective Global Assessment Physical Examination Guidance Sheet

Reference: (Shaw 2011)

Body Area	Assessing	Well nourished	Mild/moderate malnutrition	Severe malnutrition	Special tips
Head					
Orbit	Fat	Slightly bulged fat pads		Hollow look, depression, loose skin	
Temple	Muscle	Can see well defined muscle	Slight depression	Hollowing depression	Observe patient straight on, have patient turn side to side
Shoulder					
Clavicle	Muscle	Not visible in men, may be visible, not prominent in women	Some protrusion	Protruding / prominent bone Squaring of shoulder	Look for prominent bone
Shoulder (deltoids)	Muscle	Rounded curves at junction of shoulder and neck or arm	Acromium process may protrude slightly	Shoulder to arm joint looks square, bones prominent. Hollowing	Arms at side, look for prominent bones
Arm					
Triceps skinfold	Fat	Ample fat tissue		Very little space between fingers or fingers touch	Arm bent; be careful not to include muscle in pinch; roll skin between fingers
Interosseous muscle	Muscle	Muscle protrudes, could be flat in well nourished females	Slightly depressed or flat	Flat or depressed area between thumb and forefinger. Hollowing	Back of hand, move thumb and fore finger back / forth Get patient to press thumb and forefinger together
Back					
Scapula (lat dorsi, trapezius, deltoids)	Muscle	Bone not prominent, not significant depressions. May be evident but not prominent	Mild depressions or bone may show slightly	Prominent visible bone, depression between ribs, scapula and shoulder or spine	Look for prominent bones. Have patient push hands against solid object
Fat overlying lower ribs	Fat	Lower rib well covered	Lower rib visible	Depression between lower rib and back and/or between ribs	
Legs					
Thigh (quadriceps)	Muscle	Well rounded no depressions. No protrusion of		Bones prominent. Hollowing.	Not as sensitive as upper body

Body Area	Assessing	Well nourished	Mild/moderate malnutrition	Severe malnutrition	Special tips
		the knee		Look for tone and volume	and more responsive to changes in activity. Look for tone and volume
Calf (gastrocnemius)	Muscle	Well developed bulb		Thin, no muscle definition	Look for muscle tone and volume
Fluid status					
Ankles					Check for pitting oedema
Sacral oedema				Can use nurse if help required or record any existing notation in notes.	Use in bed/chair bound patients. Looking for pillow of fluid at base of spine
Ascites					Check medical notes

8.13 CCR 3703 Ethical Approval Letter for Amendment to Protocol

NRES Committee London - Riverside
Bristol Research Ethics Committee Centre
Level 3 Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT

Tel: 0117 342 1390
Fax: 0117 342 0445

27 July 2012

Ms Eva Grace
C/O Dr Andreyev, GI Unit
The Royal Marsden NHS Foundation Trust, Fulham Road
London
SW3 6JJ

Dear Ms Grace

Study title:	**An observational study to assess the prevalence and profile of oesophago-gastric cancer patients who develop small intestinal bacterial overgrowth during or after cancer treatment**
REC reference:	**11/LO/1583**
Protocol number:	**CCR3703**
Amendment number:	**1**
Amendment date:	**24 May 2012**

Thank you for submitting the above amendment, which was received on 20 July 2012. It is noted that this is a modification of an amendment previously rejected by the Committee (our letter of 13th July 2012 refers).

The modified amendment has been considered on behalf of the Committee by the Chair.

Ethical opinion

There were no ethical issues.

I am pleased to confirm that the Committee has given a favourable ethical opinion of the modified amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved are:

Document	Version	Date
Participant Information Sheet	3	17 July 2012

Modified Amendment	1	24 May 2012
Covering Letter		

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

11/LO/1583:	**Please quote this number on all correspondence**

Yours sincerely

P.P. Tina Cavalho

Dr Sabita Uthaya
Chair

E-mail: ubh-tr.riversiderec@nhs.net
Copy to: *Ms Jane Lawrence, Royal Marsden NHS Foundation Trust*
Dr Jervoise Andreyev, The Royal Marsden NHS Foundation Trust

8.14 CCR 3736 Ethical Approval Letter

NRES Committee London - Central
Level 7N019, Maternity Block
Northwick Park Hospital
Watford Road
Harrow
Middx
HA1 3UJ

Telephone: 020 8869 3775
Facsimile: 020 8869 5222

19 December 2011

Miss Eva Grace
The Royal Marsden
Fulham Road
London
SW3 6JJ

Dear Eva

Full title of study:
An observational study to develop a new method for diagnosing small intestinal bacterial overgrowth.
REC reference number:
11/LO/1870
Protocol number:
EudraCT number:
CCR 3736
N/A

Thank you for your email of 16/12/2011. I can confirm the REC has received the documents listed below as evidence of compliance with the approval conditions detailed in our letter dated 30 November 2011. Please note these documents are for information only and have not been reviewed by the committee.

Documents received

The documents received were as follows:

Document
Version
Date
Participant Information Sheet
2
08 December 2011

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor"s responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

11/LO/1870

Please quote this number on all correspondence

Yours sincerely

8.15 CCR 3736 Patient Information Sheet

‘Developing a new method for diagnosing small bowel bacterial overgrowth’

Introduction
We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Ask us if there is anything, which is unclear to you, or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for taking the time to read this.

Why have I been invited to take part?
You have been invited to take part in this study because you are a patient at The Royal Marsden NHS Foundation Trust. You are suffering from symptoms related to your cancer or the treatment you received/are receiving for it. You have been referred to one of Dr Andreyev’s clinics because of these symptoms. This study will recruit men and women who meet these two criteria. This is why you have been selected to take part.

What is the purpose of the study?
The study has objectives, which includes finding out more about a condition called small bowel bacterial overgrowth. To date, there has been very little research looking at the condition in patients undergoing treatment for their cancer, or in the aftermath of that treatment. The condition typically causes symptoms as follows; abdominal pain/discomfort, nausea, diarrhoea, constipation, bloating, excessive gas, vomiting or a change in the frequency of stools. These symptoms may develop before treatment, in the early stages of your treatment and/or continue for months or years after you have finished it. We would like to find out more about the specific symptoms which patients experience, as they can affect the patient’s quality of life.

What is small bowel bacterial overgrowth?
In most healthy people, very few bacteria are able to survive in the upper part of the small bowel. It is usually a very sterile area. Nearly all of the bacteria living along the gastrointestinal tract live in the large bowel. Their role here is very important for our health. Sometimes, often in illness, these bacteria move upwards into the small bowel, where they can multiply. If they reach large enough numbers, they start to cause symptoms e.g. diarrhoea, bloating, gasiness, weight loss and nutritional deficiencies. This is called ‘small bowel bacterial overgrowth’.

There are many reasons why small bowel bacterial overgrowth can occur in patients with cancer. It may develop following surgery, where the anatomy of the

gastrointestinal tract has been changed. Patients who receive chemotherapy and/or radiotherapy may also be at risk of developing it because the healthy cells of the gastrointestinal tract may become damaged and so bacterial numbers can increase. We hope that this study will provide us with more information about the condition and this will allow early diagnosis of it and so quicker improvement in the symptoms that it causes.

Do I have to take part?
It is up to you whether or not you decide to join this study. We will describe the study and go through this information sheet with you. If you agree to take part we will then ask you to sign a consent form. You are free to withdraw at any time without giving a reason. Your usual treatment will not be affected in any way.

What will happen to me if I take part?
If you decide to take part you will be seen by the research dietitian who is running the study– she will be in touch with you during the study, which will last for 3-months. You will see her when you are at The Royal Marsden Hospital for the appointments, which Dr Andreyev has made/will make for you. You will not have to make any additional trips to the hospital outside of your normal clinic appointments. However, we will record the information gathered at these appointments.

Dr Andreyev will ask you to carry out a glucose breath test. It is a simple, low risk procedure, which is used to get an idea of the number of bacteria in your small bowel by measuring the gases in your breath. It helps with diagnosing small bowel bacterial overgrowth. The test will take place in the morning and you will be asked to have been fasting since the night before. The dietitian (or a nurse) will give you a sugary liquid and will ask you to drink it. You will then breathe into a bag once every 20 minutes for the next 1.5 hours and your breath will be analysed using a machine.

At this appointment, you will be asked to fill in a questionnaire, which can be done during the breath test. It will ask you about your gastrointestinal symptoms. On the day of the appointment, the dietitian will collect two pots from you; one containing a urine sample and one containing a stool sample. You will have been provided with the pots prior to your appointment.

Dr Andreyev may make an endoscopy appointment for you too. This is to allow him to take a look down your gullet and into your gastrointestinal tract. As a part of the procedure, a sample of the fluid in the tract is collected. If this test is done, some additional fluid will also be collected for our study. The fluid collected, as well as the urine and stool samples will be used to help with developing a new diagnosis for small bowel bacterial overgrowth.

When you return to Dr Andreyev's clinic for a review appointment, you will see the dietitian again. She will ask you to complete the same symptom questionnaire as before. She will also collect a urine sample and a stool sample from you in pots, which she has previously provided you with.

What are the possible risks and disadvantages of taking part?
Taking part in this study poses no additional risk to you than if you refuse to take part. This is because the only extra requirement would be for you to complete some questionnaires and to provide samples of urine and stool. The breath test and endoscopy appointment are a part of routine clinical practice.

What are the possible benefits of taking part?
There is one main benefit of the study. We hope the study may help patients in the future, as it will allow us to gather invaluable data, which in the longer term may allow us to develop a new test for diagnosing small bowel bacterial overgrowth. This will allow us to deliver improved care to them.

What happens when the research study stops?
Your normal care will continue in the usual way.

Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be kept confidential. If you consent to take part in the study, you will receive a study number so that none of the information gathered about you can be traced back to you. There will be other organisations involved in the study also. This is because a special technique (called NMR spectroscopy) is needed to help us analyse the samples which you provide. These organisations include King's College London, the University of Reading and Korrigan Sciences Limited. We will ensure that all data is kept completely confidential at each step along the way.

Who is organising and funding the research?
The Royal Marsden NHS Foundation Trust is sponsoring this research.

Who has reviewed the study?
The Royal Marsden Committee for Clinical Research (CCR) and the London Central National Research Ethics Committee.

Further Information
Before you make a decision about your participation in this study, remember that you can ask us any questions. Allow yourself as much time as you need to think through your decision. If you then decide that you still wish to take part, you will be asked to confirm in writing that you have read and understand this patient information and that all of your questions have been answered completely and that you wish to continue in the study. If you would like further information about the study, or have any concerns during the study, please contact Ms Eva Grace, Research Dietitian, Tel. 020 7352 8171 (ext. 4653) or Dr Jervoise Andreyev, Consultant Gastroenterologist in Pelvic Radiation Disease, Tel. 020 7811 8216, who will be happy to discuss the study with you.

8.16 CCR 3736 Consent Form

The ROYAL MARSDEN
NHS Foundation Trust

Study Protocol Number:
Ethics Protocol Number:
Patient Identification Number for this trial:

Study title: "An observational study to develop a new method for diagnosing small intestinal bacterial overgrowth" (SIBO II Study).

Name of Principal Investigator: Dr. Jervoise Andreyev

Please initial box

1. I confirm that I have read and understand the patient information sheet (version 3) dated 2nd October 2012 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and my medical care and legal rights will not be affected. ☐
3. I give permission for individuals from regulatory authorities, from The Royal Marsden and the other relevant organizations as mentioned in the patient information sheet (version 3, dated 2nd October 2012), to have access to relevant sections of my medical notes and data (including samples) collected during the study. ☐
4. I consent to the researcher keeping the anonymous data in a locked office for a maximum of fifteen years post commencement of the study for confirmation purposes during preparation for publication. ☐
5. I have been assured strict confidentiality will be maintained. ☐
6. I agree to take part in the above study. ☐
7. I would/ would not like to be informed of the results of this study. *Please circle one*.

Please sign here:

----------	----------	----------
Signature	Date	Name of Participant
----------	----------	----------
Signature	Date	Name of Researcher

8.17 Instructions for Stool and Urine Sample Collection

Dear _____

Thank you very much for agreeing to take part in the SIBO II study. As discussed, I would like you to provide me with a stool sample and a urine sample. Ideally the samples should be collected **on the day of** your endoscopy appointment with Dr Andreyev.

Appointment Date:

Appointment Time:

I have enclosed some stool and urine sample collection items and have provided instructions below.

Instructions for **stool** collection:

1) Please wear a pair of disposable gloves.
2) Use the card tray to collect the stool before it drops into the pan.
3) Decant enough stool to fill the yellow-top container (labelled ‘stool’) using the wooden spatula.
4) Dispose of any surplus stool and collection items.

Instructions for **urine** collection:

1) The sample can be collected at any time.

2) Please wear a pair of disposable gloves.
3) Start to urinate but don't collect the first part of urine that comes out i.e. collect a mid-stream sample.
4) Screw the lid of the yellow-top container (labelled 'urine') shut.

Note:
Please fill up the yellow-topped containers as otherwise we will not have enough of the samples to perform a complete analysis.

Finally…

1) Place each of the yellow-top containers in a separate clear bag and seal each bag.
2) Put both of these bags into the white carrier bag.
3) Bring the samples with you when you come to your endoscopy appointment and please ask one of the endoscopy nurses to call me when you arrive (ext. 4653).

I look forward to seeing you and many thanks again.

With best wishes,

_____

Eva Grace

Research Dietitian
Tel: 0207 352 8171 (ext. 4653)